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COMPARATIVE STUDIES OF CERTAIN CLADOSPORIUM DISEASES OF STONE FRUITS

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In consequence of its wide distribution and of the serious losses which it occasions, peach scab (caused by *Cladosporium carpophilum* Thüm.) has been the subject of numerous investigations. Similar diseases of other stone fruits have long been observed, but have received less study. Doubt still exists, therefore, concerning the interrelationships of the Cladosporium diseases of the various stone fruits and of the organisms which cause them. During a sojourn of the senior author at the University of Wisconsin, the present writers undertook some comparisons of those Cladosporium diseases of stone fruits of which suitable material was available (See preliminary report by Bensaude and Keitt, 2). A brief account of this work[1] follows.

SYMPTOMS

The symptoms of peach scab are well known, having been described and illustrated by many writers (See Keitt, 3, p. 6–8, Pls. I–IV). The previous accounts of the Cladosporium diseases of plum, apricot, and cherry, however, have been somewhat less complete, and are, therefore, supplemented by the following descriptions.

Plum scab

On fruit.—At Madison, Wis., in 1922 the first macroscopic evidence of scab was observed on fruits of *Prunus americana* Marsh. about June 15, when the young plums were approximately 8 millimeters in diameter. The typical lesion first appeared as a small, circular, olivaceous patch, the color of which was due chiefly to the fruiting of the causal fungus. Very soon,

[1] Publication of these results, which were limited in scope by the time available, has been delayed in the hope that the junior author might supplement them by further studies. As this has not yet been feasible, it seems desirable to report the available results without further delay.

however, the epidermal and hypodermal host cells under the olivaceous area and in a narrow zone about it developed a yellowish color. Later, as suberization progressed, the central part of the lesion took on a brown, corky aspect. As the fruit began to color, a red halo commonly appeared around the lesion. On the dark carmine, ripe fruit the lesions were much like the surrounding surface in color, except for lack of gloss and for the olivaceous or brown aspect of the fungus and of the suberized area. At this stage the spots frequently had a slightly raised border, and were rather sharply defined. In the later stages of the development of the lesions, the fungus commonly continued to grow in its subcuticular position, forming a pseudoparenchymatous structure from several to many cells in thickness. In the period of rapid fruit growth prior to ripening, the lesions in which cork development had been most marked commonly cracked. At any stage after the lesions became macroscopic, conidiophores and conidia could be found upon the surface.

Lesions were found to occur commonly on the pedicels. Before they became macroscopically evident, the conidial fructification of the fungus could be detected by use of a hand lens. As the growth of the fungus became more dense, it gave a grayish olivaceous aspect to the diseased area. Later, as the pedicels became yellowish, the lesions became suberized and brown, and were clearly delimited. Conidiophores grew abundantly on these lesions, and commonly bore an abundant crop of conidia when held over night in a moist chamber. Sometimes old lesions on the pedicels sloughed off late in the season.

On twigs.—The lesions on twigs were of the same general type as those on peach twigs (Keitt, 3, p. 7), except that they were commonly smaller, probably due to the fact that the wild plum twigs examined had made a much less vigorous growth than those of well cultivated peach trees.

On leaves.—The leaves of *P. americana* did not appear to be very susceptible to injury by *Cladosporium*. The symptoms on the leaves were not conspicuous. The chief macroscopic evidence of infection was a slight brown or olivaceous discoloration, which usually occurred on the midribs or veins on the lower (dorsal) surface. Conidiophores and conidia were found on these discolored areas. Infection occurred quite commonly on the petioles, where the lesions much resembled those which develop on the pedicels.

Apricot scab

The only lesions on *Prunus armeniaca* L. available for examination were those induced on twigs and leaves by inoculation.

On twigs.—The lesions first appeared as reddish flecks, which later became brown. Their development closely paralleled that of lesions on twigs of peach and plum.

On leaves.—Infection was always more abundant on the lower (dorsal) surface, though conidiophores and conidia have been observed to occur sparsely on the upper surface. The very young lesions were commonly angular in outline, conforming with the areas bounded by the smallest veinlets. At first pale, olivaceous yellow, they commonly became more yellow with age. On the parts of the leaf more exposed to the sun they often took on a bright red or purplish tint. The lesions were found to develop on the lamina or on the veins, midrib, or petiole. When they occurred in sufficient abundance, they frequently became confluent. When fully developed they ordinarily varied from one to several millimeters in diameter.

Cherry scab

Scab was observed on unsprayed, neglected Early Richmond trees (*Prunus cerasus* L.) at Sturgeon Bay, Wis.

On fruit.—The first evidence of fruit infection was observed as barely macroscopic pink splotches, which had a stippled appearance under the hand lens because of unequal reddening of the sap of the cells of the affected areas. As the lesions, which were unchanged in elevation and imperfectly demarked from the surrounding tissue, enlarged slowly, the color of the middle area faded to a dull red or brown, while the brighter red continued to be developed in the advancing peripheral zone, which was usually about one-half millimeter wide, and sharply defined at the inner margin. The coloration of the lesions varied somewhat with the stage of development of the fruit. The fully developed lesions varied from one-half to two millimeters in diameter. They showed no change in elevation or hardening of tissue. They usually bore conidia of the causal fungus rather sparsely when freshly collected from nature, but in abundance after being held over night in a moist chamber. As many as 40 lesions were found on a single badly diseased fruit.

On twigs.—The first macroscopic evidence of twig infection appeared as small, irregular to oval, imperfectly defined, brownish areas about one-half millimeter in greatest dimension. The color at this stage is due chiefly to the presence of conidiophores and conidia of the causal fungus. The fully developed lesions were usually irregular to oval, and little changed in general aspect, though they sometimes took on a brown color and became very slightly elevated.

On leaves.—The lesions first appeared on the upper (ventral) surface of green leaves as barely visible flecks, slightly lighter than the normal green. At this stage it was necessary to secure very favorable illumination in order to see them. They were irregular to circular in outline, unchanged in elevation, and bore abundant conidia of the causal fungus. No

lesions larger than one to two millimeters in diameter were observed. The amount of material examined, however, was limited. On leaves which were yellowing, the infected areas and a peripheral zone commonly held their green color longer than did the surrounding tissue. No lesions were observed on the under (dorsal) surface of the leaves.

MODE OF PENETRATION OF THE HOST BY CLADOSPORIUM CARPOPHILUM

In preliminary experiments it was found that the leaves of apricot are very susceptible to infection by *C. carpophilum* and that potted apricot plants thrive well under greenhouse conditions. Consequently, this host (variety Superba) was chosen for studies of the mode of penetration of leaves by the fungus.

The inoculum consisted of a suspension in sterile distilled water of spores from single-spore cultures of a strain of *Cladosporium* which had been isolated at Madison, Wis., from *P. americana* and grown on Lima bean agar. The cultures used were fresh (usually 8 to 12 days after transfer) and bore abundant conidia. The viability of the spores was tested by placing droplets of the inoculum on clean glass slides which were incubated in a moist chamber at approximately 22° C. Ordinarily, from 80 to 100 per cent germinated within 48 hours.

Drops of the inoculum were placed on marked areas on the lower (dorsal) surface of the leaves. They were permitted to dry, and other drops were added. The potted plants were then placed in a large moist chamber in which the temperature was approximately 22° C. and the relative humidity was approximately 100 per cent. After three or four days the plants were placed either in a well-illuminated chamber in which the temperature was approximately 22° C. and the relative humidity 85 per cent, or out of doors. On the second day and at intervals thereafter, leaves were collected and examined. To facilitate examination the following method was used. The inoculated areas were placed for 6 to 12 hours in a solution consisting of equal parts by volume of glacial acetic acid and 95 per cent alcohol. They were then cleared for one to several days in a saturated aqueous solution of chloral hydrate. They were then immersed for 6 to 12 hours in Pianeze's stain (Vaughan, 6), after which they were cleared in carbol turpentine for a few hours. In successful preparations the spores or germ tubes on the surface of the leaves were stained purple or blue, whereas the subcuticular mycelium was colored in magenta or pink. The epidermal cells of the leaves were stained a bright green. If a permanent mount was desired, the leaf fragments were rapidly dehydrated, washed in clove oil and xylol, and mounted in Canada balsam. After this treatment, the spores and surface mycelium were red instead of purple, while the other

parts which have been mentioned were not considerably changed in color if immersion in alcohol was not prolonged.

Examinations made on the second day after inoculation commonly showed that a high percentage of the spores had germinated. The germinating spores produced from one to three germ tubes each. Some of these tubes were comparatively long. These failed to develop infection hyphae. The tubes from which penetration was observed to occur were comparatively short, measuring from 7 to 35 microns in length. With the technique used, however, no penetration was observed in material collected on the second day. The material of the second collection, which was made four days after inoculation, showed abundant infection. Sufficient subcuticular development had occurred to suggest that penetration had taken place considerably before this collection was made. From field observations and from experience with other closely related parasites it seems probable that penetration occurs in a much shorter time than four days.

In the material studied penetrating hyphae were observed in large numbers. No well-defined appressoria were seen. Penetration occurred at or near the apex of the germ tube, the circular lumen of the penetrating hypha being visible as a delicate circular orifice (Figs. 1, 2). A slightly lower focus often revealed the subcuticular mycelium. In the type of preparation studied the detailed structure of this mycelium was not sharply defined. In the early stages following penetration, it often appeared as a small, some-

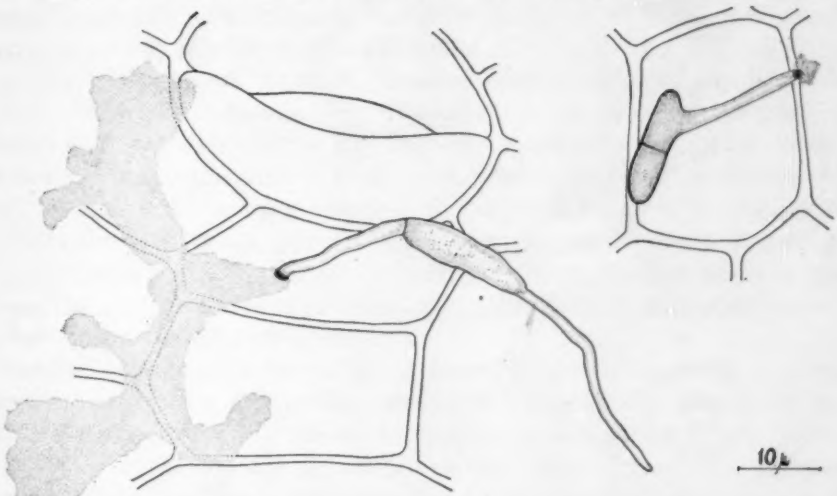

FIG. 1. *Cladosporium* from *P. americana* parasitizing the dorsal surface of a leaf of *P. armeniaca*, four days after inoculation with conidia. Camera lucida drawing of material stained *in toto*. The stippled strands show the general distribution of the subcuticular mycelium.

what amoeboid rosette about the point of entry of the penetrating hypha. With further growth, flattened ribbon-like strands of subcuticular mycelium developed, frequently seeming to follow roughly the outlines of the epidermal cells. As has been reported for various other parasitic fungi, penetration appeared to occur more frequently near the boundaries of epidermal cells than over the middle portions of these cells. At the time when the lesions first became macroscopically evident, these flattened ribbon-like strands of subcuticular mycelium had ramified the invaded areas. Conidiophores were borne profusely from this mycelium, penetrating the cuticle freely, whether they were developed near the boundaries of epidermal cells or over the middle portions.

A more detailed record of the processes of infection should be revealed by studies of sections prepared by the usual histological methods. Such work was contemplated, but could not be completed in the time available.

CROSS-INOCULATION EXPERIMENTS

The inoculum used consisted of suspensions in sterile distilled water of spores and mycelial fragments taken either from sporulating single-spore

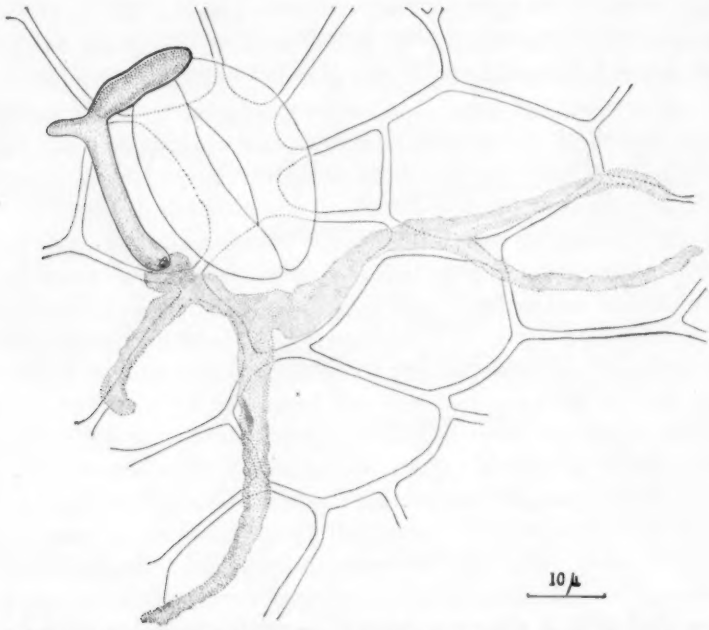

FIG. 2. *Cladosporium* from *P. americana* parasitizing the dorsal surface of a leaf of *P. armeniaca*, nine days after inoculation with conidia. Camera lucida drawing of material stained *in toto*.

pure cultures on Lima bean agar or from sporulating lesions on leaves or fruits of naturally infected *Amygdalus persica* L., *P. americana,* or *P. cerasus.* The species inoculated were *A. persica* (Summer Heath), *P. armeniaca* (Superba), *P. americana* (seedlings of native wild plum), *P. cerasus* (Montmorency and Early Richmond), and *P. domestica* L. (Lombard). In some tests the plants were in pots in the greenhouse; in others, they were in plantings out of doors.

The inoculum was applied to marked twigs and to both surfaces of the leaves by means of sterile atomizers. After inoculation the potted plants were placed in a large moist chamber at temperatures of 18 to 23° C. for three days and then on a greenhouse bench or out of doors, depending upon the season. Plants growing out of doors were inoculated in a similar manner and kept moist for three days by the apparatus described by Keitt (4, p. 546–547). An appropriate number of uninoculated plants of each species studied served as controls. The experimental plants were examined and noted at suitable intervals. The results appear in table 1.

The *Cladosporium* from *A. persica* induced abundant infection on the leaves and twigs of *A. persica* and *P. armeniaca,* and slight infection on the leaves of *P. americana.* No evidence of infection appeared upon *P. cerasus* or *P. domestica* inoculated with the fungus from the peach.

The fungus from *P. americana* induced infection upon the leaves of *P. americana* and *A. persica* and the leaves and twigs of *P. armeniaca.* No evidence of infection appeared upon the plants of *P. domestica* and *P. cerasus* inoculated with the plum fungus.

The *Cladosporium* from *P. cerasus* induced infection on leaves of *P. cerasus* (Early Richmond). Flecks were several times observed on leaves of *P. armeniaca* which had been inoculated with the cherry fungus. However, no fructification of *Cladosporium* was ever found on these flecks. No lesions or flecks were observed to follow inoculation of the cherry *Cladosporium* upon *A. persica.* However, in one instance five or six conidiophores and a few conidia which appeared to be *Cladosporium* were found on one peach leaf thus inoculated. The identity and significance of these were not clearly established.

From these data it appears that strains of *Cladosporium* from *A. persica* and *P. americana* are pathogenic on *A. persica, P. americana,* and *P. armeniaca. P. cerasus,* however, appears to be highly resistant to these strains. The *Cladosporium* from *P. cerasus* induced no typical infection on any of the host plants on which it was tried, except *P. cerasus.*

None of the control plants showed any evidence of disease. To conserve space, the data from these plants are omitted from table 1.

Observations were made with the aim of determining whether failure to effect cross-inoculations in certain instances was due to inability of the

TABLE 1.—*Summary of results of cross-inoculation experiments with Cladosporium spp. on stone fruits, Madison, Wis., 1921–1922*

Location and date of inoculation	Inoculum		Plant inoculated	Minimal period of incubation (days)		Infection
	Host from which obtained	Source		On leaves	On twigs	
In greenhouse						
May 18, 1922	*Amygdalus persica*	19-day-old culture	*Amygdalus persica*	.....	.....	None
do	do	do	*Prunus armeniaca*	31	.....	Slight
do	do	do	*P. cerasus* (E. R.)	.....	.....	None
July 21, 1922	do	11-day-old culture	*A. persica*	64	.....	Abundant
do	do	do	*P. cerasus* (E. R.)	.....	.....	None
Out of doors						
July 19, 1921	do	20-day-old culture	*A. persica*	15	22	Abundant
do	do	do	*P. armeniaca*	16	30	do
do	do	do	*P. domestica*	.....	.....	None
do	do	do	*P. americana*	39	.....	Slight
Aug. 10, 1921	do	14-day-old culture	*P. domestica*	.....	.....	None
do	do	do	*P. americana*	52	.....	Slight
do	do	do	*P. cerasus* (E. R.)	.....	.....	None
July 21, 1922	do	do	*A. persica*	33	.....	Abundant
do	do	do	*P. armeniaca*	31	.....	do
In greenhouse						
May 25, 1922	*Prunus americana*	Culture	*A. persica*	.....	.....	None
do	do	do	*P. armeniaca*	24	28	Abundant
do	do	do	*P. cerasus* (E. R.)	.....	.....	None
June 1, 1922	do	do	*A. persica*	86	.....	Fair
do	do	do	*P. armeniaca*	23	.....	Abundant
do	do	do	*P. cerasus* (E. R.)	.....	.....	None
June 21, 1922	do	4-day-old culture	*P. armeniaca*	10	.....	Abundant
June 24, 1922	do	Culture	do	13	.....	do
July 8, 1922	do	10-day-old culture	do	30	.....	Slight

TABLE 1.—(*Continued*)

July 8, 1922	do	10-day-old culture	do	30	.....	Slight

TABLE 1.—(*Continued*)

Location and date of inoculation	Inoculum		Plant inoculated	Minimal period of incubation (days)		Infection
	Host from which obtained	Source		On leaves	On twigs	
In greenhouse	*P. americana*					
July 13, 1922	do	12-day-old culture	*P. armeniaca*	27	.....	Abundant
July 21, 1922	do	4-day-old culture	*A. persica*	.....	.....	None
do	do	do	*P. armeniaca*	62	.....	Slight
do	do	do	*P. cerasus* (E. R.)	.....	.....	None
Out of doors						
Aug. 2, 1921	do	Lesions	*A. persica*	44	.....	Slight
do	do	do	*P. armeniaca*	41	.....	Abundant
do	do	do	*P. domestica*			None
do	do	do	*P. americana*			None
June 19, 1922	do	16-day-old culture	*P. armeniaca*	25	.....	Abundant
do	do	do	*P. americana*	25	.....	Slight
do	do	do	*P. cerasus* (E. R.)	.....	.....	None
June 29, 1922	do	9-day-old culture	*A. persica*	.....	.....	None
do	do	do	*P. americana*	18	.....	Slight
do	do	do	*P. cerasus*	.....	.....	None
In greenhouse						
April 27, 1922	*P. cerasus*	14-day-old culture	*A. persica*	.....	.....	None
do	do	do	*P. armeniaca*	.....	.....	None
do	do	do	*P. cerasus* (M.)	.....	.....	None
do	do	do	*P. cerasus* (E. R.)	58	.....	Slight
May 9, 1922	do	10-day-old culture	*A. persica*	.....	.....	†[a]
do	do	do	*P. armeniaca*	.....	.....	None
do	do	do	*P. cerasus* (M.)	.....	.....	None
do	do	do	*P. cerasus* (E. R.)	15	.....	Abundant
July 14, 1922	do	16-day-old culture	*P. armeniaca*	.....	.....	None
do	do	do	*P. cerasus* (E. R.)	26	.....	Abundant

[a] No macroscopic lesion was developed. Microscopic examination revealed one group of a few conidiophores and conidia which resembled *Cladosporium*.

fungus to penetrate the host or to other reasons. Application of the technique described earlier showed clearly that the cuticle of the leaves of *P. cerasus* was commonly penetrated by the *Cladosporium* from *P. americana*, though little development of the fungus occurred after penetration. The cuticle of leaves of *P. armeniaca* was similarly penetrated by the fungus from *P. cerasus*. Slight flecking of the leaves but no sporulation of the fungus followed this inoculation. These results are very similar to those reported by Wiltshire (6) for *Venturia inaequalis* (Cke.) Wint. and *V. pyrina* (Cke.) Aderhold on apple and pear.

RELATIONS OF TEMPERATURE

Conidia from pure cultures of *Cladosporium* from *P. americana* and *P. cerasus*, respectively, were suspended in sterile distilled water. Drops of these spore suspensions were placed on the surfaces of agar (2 per cent agar in water) in petri dishes, which were incubated in dark chambers at a range of constant temperatures (variable 1° C.). After 24 hours, data were taken on the percentage of spores germinated and, in certain series, on the length of germ tubes. The results are shown graphically in figures 3 and 4. The data shown in figure 3 are averaged from four series, in each of which the records were made from 50 spores chosen at random in each plate. The results shown in figure 4 were taken similarly from two series, being based

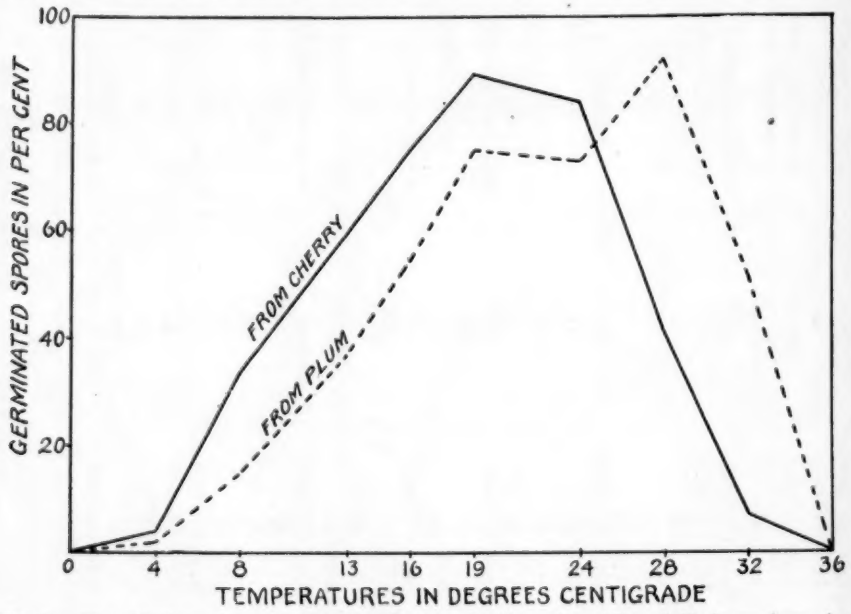

FIG. 3. The relation of temperature to the germination of conidia of Cladosporiums from cherry (*P. cerasus*) and plum (*P. americana*).

on measurements of the length of the germ tubes of ten germinated spores in each plate. These data suggest that the *Cladosporium* from *P. americana* has a perceptibly higher range of temperature for conidial germination than that from *P. cerasus*. The records in figure 4 indicate that, under the conditions of these experiments, the germ tubes of the cherry fungus elongate more rapidly than do those of the plum *Cladosporium*.

Drops of spore suspensions of the type just described were placed on the surfaces of Lima bean agar in petri dishes which were then incubated at a range of constant temperatures (variable 1° C.). In each series it was sought to have the drops as nearly uniform as feasible in size and in the area of their distribution on the agar. After 10 days, observations were made on the macroscopic aspects of the fungal growth. No significant difference was observed in the development of strains of *Cladosporium* isolated, respectively, from *P. americana* and *A. persica*. With each of these strains, little macroscopic evidence of growth appeared at temperatures below 16° C. The most vigorous growth occurred within the range of 19–28° C. Good, but less vigorous, growth occurred at 31–33° C., the highest temperature tried. In so far as macroscopic appearance is a criterion of growth, the *Cladosporium* isolated from *P. cerasus* seemed to have a distinctly lower temperature range on the medium used. In this case, good growth oc-

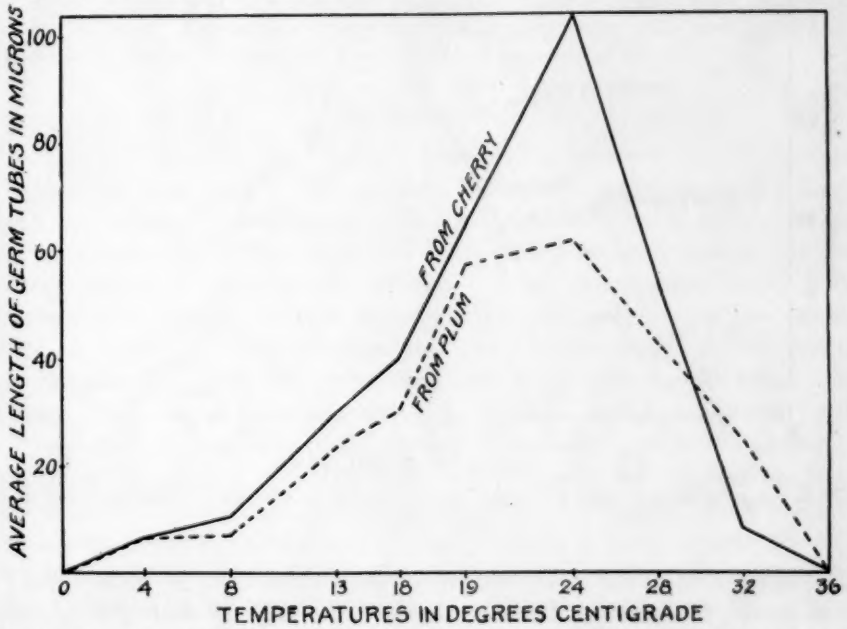

FIG. 4. The relation of temperature to the length of germ tubes developed by conidia of Cladosporiums from cherry (*P. cerasus*) and plum (*P. americana*).

curred at 13–16° C. The most vigorous development was observed in the range of 19–26° C. At 28–31° C. growth was much retarded. At 31–33° C. no macroscopic evidence of growth was observed.

While these data are too limited to be fully conclusive, they strongly suggest that the *Cladosporium* which occurs on *P. cerasus* has a somewhat lower thermal range for germination and growth than have the strains which parasitize *P. americana* and *A. persica.*

MORPHOLOGY

Comparative studies of conidia and conidiophores revealed no sharply defined morphological differences in the Cladosporiums on *A. persica, P. americana,* and *P. cerasus.* The material studied conformed closely with the descriptions by von Thümen (4, p. 13), Aderhold (1, p. 542), and Keitt (3, p. 12–14). Measurements were made of the lengths of 300 conidia from lesions on fresh material from each of these hosts. The spores were mounted under a thin cover slip on a clear agar gel and measured promptly under an oil immersion lens. The results, which appear in figure 5, show a

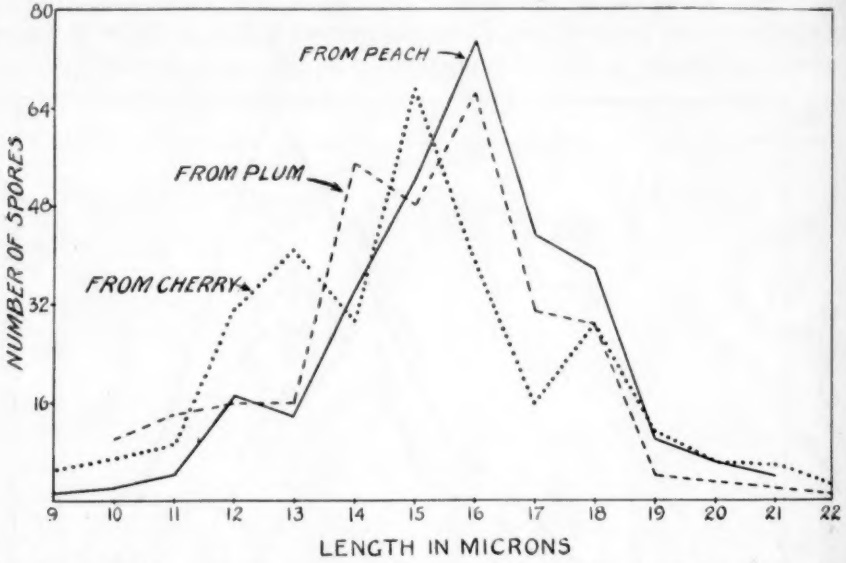

FIG. 5. A graphic summary of length measurements of conidia of Cladosporiums from field material.

close agreement in the measurements of the strains from *A. persica* and *P. americana.* The conidia from *P. cerasus* were slightly shorter than those from the other two hosts. The data, however, are not sufficiently extensive to indicate clearly whether or not this difference is significant. As is shown

in table 2, it was lessened when the conidia measured were taken from cultures grown according to a standardized method. Further studies will be necessary to determine whether or not the suggestions of bi- and trimodality in the polygons of the plum and cherry strains are significant. The conidia which were measured varied in width from 3.5 to 6 microns, the most common range extending from 4 to 5.5 microns.

TABLE 2.—*Length of conidia of Cladosporiums from certain stone fruits*[a]

Source of conidia	Number measured	Average length in microns
Lesions on fruit of *Amygdalus persica*	100	16.3
Lesions on fruit of *Prunus americana*	100	16.2
Lesions on fruit of *P. cerasus*	100	15.6
Culture from *A. persica*	100	18.9
Culture from *P. americana*	100	19.1
Culture from *P. cerasus*	100	18.2

[a] The measurements of spores from lesions were made in August from freshly collected specimens. The cultures from which measurements were made were grown comparatively on potato dextrose agar slants in a dark chamber at 23° C.

CULTURAL STUDIES

Strains of *Cladosporium* from *A. persica*, *P. americana*, and *P. cerasus*, respectively, were grown comparatively on several media, including Lima bean and potato dextrose agars. No significant differences were observed in the development of the peach and plum strains on these media. In the earlier stages of development of thalli from germinating spores, the *Cladosporium* from *P. cerasus* varied distinctly from the other two strains (Figs. 6, 7, and 8). The conidia of this strain showed little swelling during germination, and gave rise to comparatively long, slender germ tubes. In the stages of development represented in figures 7 and 8, the young thalli of the cherry fungus were distinctly less abundantly septate than those of the other two strains, the cells were less torulose, and the conidia from which the thalli developed maintained their identity. The young thalli of the strains from *A. persica* and *P. americana* were more abundantly septate and branched, while the swollen conidia soon lost their identity among the torulose cells which adjoined them. The thalli of these two strains tended to produce conidia earlier than did those of the cherry fungus. In the earlier macroscopic stages of development on the culture media used,

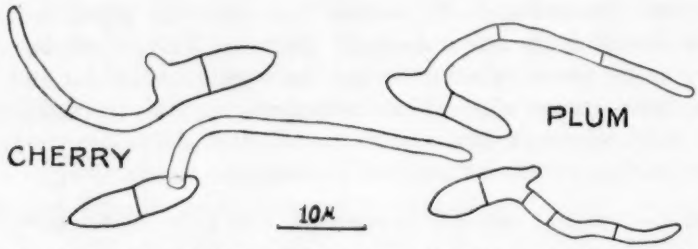

FIG. 6. Young thalli of Cladosporiums from cherry (*P. cerasus*) and plum (*P. americana*) 36 hours after conidia were placed on plates of 2 per cent agar in water at 23° C. Traced from photomicrographs.

the cherry fungus grew somewhat less compactly than the others, and showed rather more aerial mycelium. This difference lessened as the cultures grew older.

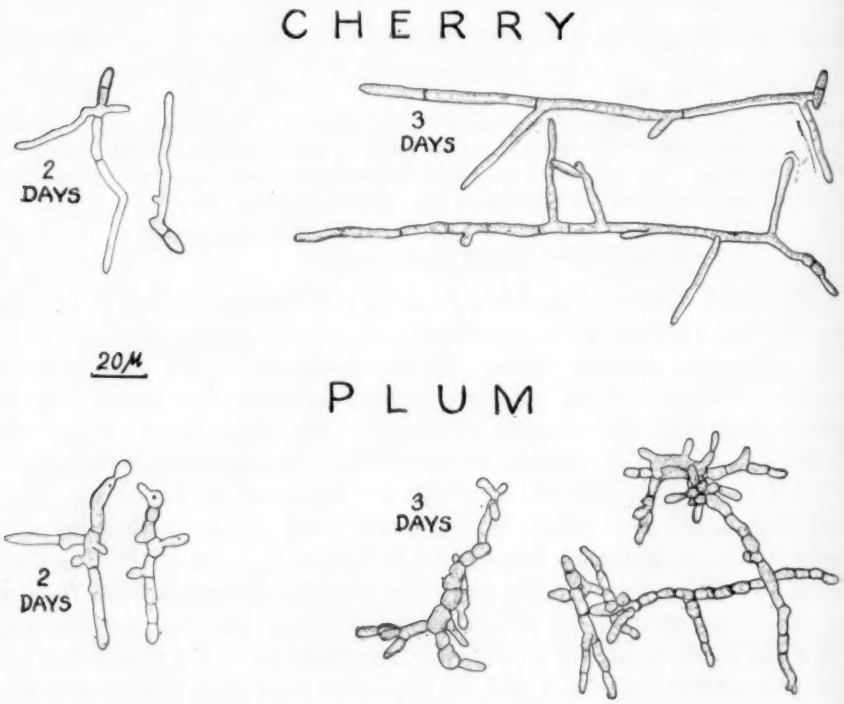

FIG. 7. Young thalli of Cladosporiums from cherry (*P. cerasus*) and plum (*P. americana*) after stated periods of growth on plates of 2 per cent agar in water at 24° C. Traced from photomicrographs.

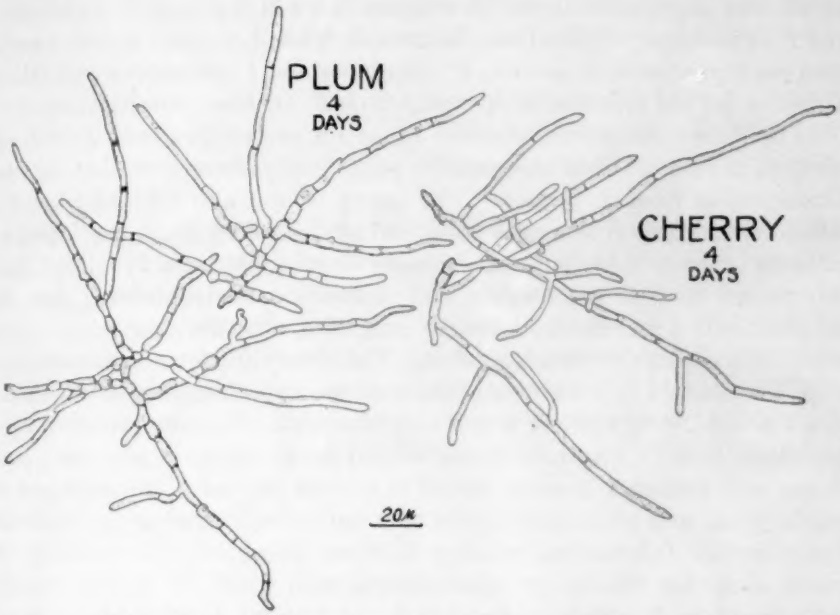

FIG. 8. Young thalli of Cladosporiums from plum (*P. americana*) and cherry (*P. cerasus*) grown on plates of 2 per cent agar in water at 24° C. Traced from photomicrographs.

DISCUSSION

The results herein reported furnish further evidence of the general similarity of the Cladosporium diseases studied. In each case the fruit, leaves, and twigs of the host plant are attacked, the general relations of host and parasite appear to be similar, and the lesions produced differ only in detail. Certain differences are reported, however, which seem to segregate these diseases into two groups: (*1*) scab of *A. persica* and *P. americana* (and probably other hosts to which these studies were not extended), and (*2*) scab of *P. cerasus*. From the ease with which *P. armeniaca* was infected with strains of the fungus from *A. persica* and *P. americana*, it seems most likely that the apricot disease falls in the group with peach and plum scab.

In these studies no significant differences have been observed in the Cladosporiums from *A. persica* and *P. americana* or in the symptoms which they induced.

The cherry *Cladosporium* differed from that of peach and plum in several respects. In the cross-inoculation experiments it induced the disease only on *P. cerasus*, giving negative results (save for some penetration of the

cuticle and slight subcuticular development) on *A. persica*, *P. americana*, and *P. armeniaca*. In contrast, the strains from *A. persica* and *P. americana* readily infected *A. persica*, *P. americana*, and *P. armeniaca*, but failed to induce typical infection or sporulation on *P. cerasus*. Under the conditions of these tests the temperature range for germination and growth of the fungus from *P. cerasus* seemed to be distinctly lower than that for the *Cladosporium* from *A. persica*. The cherry fungus also differed from the others in the form of the germ tubes and of the young thalli developed in cultures. While these data seem to show clearly that these fungi fall into two groups in their pathological and physiological relationships, they do not constitute a satisfactory basis for judgment as to the genetic and taxonomic relationships of these parasites. The cherry *Cladosporium* conforms with Aderhold's (1, p. 542) description of the imperfect stage of *Venturia cerasi* Aderh., with which it is probably identical. Since ascigerous stages have been found for certain closely related fungi, as the apple, pear, and cherry scab parasites, it seems logical to suspect that the Cladosporiums of peach, plum, and other stone fruits may have similar genetic connections. Until further information relating to these possibilities is available, it would seem that the cherry *Cladosporium* with which we worked should be referred to *Venturia cerasi* Aderh. (synonym: *Cladosporium cerasi* (Rbh.) Sacc.), and the strains from *A. persica* and *P. americana* to *Cladosporium carpophilum* Thüm.

SUMMARY AND CONCLUSIONS

1. The symptoms induced by species of *Cladosporium* on *P. americana*, *P. armeniaca*, and *P. cerasus* are described.

2. The Cladosporiums studied infect the leaves of their hosts by direct penetration of the cuticle by infection hyphae from closely appressed and strongly adherent germ tubes.

3. In cross-inoculation experiments, strains of *Cladosporium* from *A. persica* and *P. americana* infected *A. persica*, *P. americana*, and *P. armeniaca*, but failed to infect *P. cerasus* or *P. domestica*. The *Cladosporium* from *P. cerasus* infected its own host, but failed to induce typical infection or sporulation on *A. persica*, *P. americana*, or *P. armeniaca*. Infection hyphae from germ tubes of the *Cladosporium* from *P. americana* readily penetrated the cuticle of leaves of *P. cerasus*, but failed to develop sufficiently to fructify or to induce macroscopic lesions. Similar cases of penetration were observed in other cross-inoculation trials where negative results were obtained.

4. The *Cladosporium* from *P. cerasus* appears to have a distinctly lower thermal range for germination of conidia and for vegetative development than has the *Cladosporium* from *P. americana*.

5. The *Cladosporium* from *P. cerasus* differed from the strains from *A. persica* and *P. americana* in the form of germ tubes and of young thalli developed in cultures.

6. Measurements of conidia of strains of *Cladosporium* from *A. persica* and *P. americana* agreed very closely. The conidia of the *Cladosporium* from *P. cerasus* were slightly shorter, but the significance of this difference is doubtful.

7. Pathologically and physiologically, the Cladosporiums studied appear to fall into two groups: (*1*) those from *A. persica* and *P. americana*, and (*2*) that from *P. cerasus*. Their genetic and taxonomic relationships are likely to be somewhat doubtful until more is known of their possible aseigerous stages. Meanwhile, the fungi from *A. persica* and *P. americana* are tentatively regarded as identical and are referred to *Cladosporium carpophilum* Thüm.; and the cherry fungus is tentatively referred to *Venturia cerasi* Aderh. (synonym: *Cladosporium cerasi* (Rbh.) Sacc.).

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A STUDY OF PATHOGENIC AND NON-PATHOGENIC STRAINS OF PSEUDOMONAS TUMEFACIENS SM. & TOWN.

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INTRODUCTION

The longevity, distribution, and overwintering of *Pseudomonas tumefaciens* Sm. and Town. in soils of different types is a matter of great economic importance, although there is comparatively little known about any of the activities of the organism in the soil. This lack of knowledge is probably due to the difficulties involved in readily recovering the crown gall organism from soils. The method of detecting a particular pathogene in the soil by gross inoculation of soil suspensions into susceptible hosts is open to serious criticism. The development of a method by which *Ps. tumefaciens* may be readily isolated from soils has made it possible to accumulate much new information about the activity of the crown gall organism in the soil.

Riker (10), Reddick and Stewart (9), and Muncie (6) have reported work on one or another phase of this problem under natural and artificial conditions in different types of soils, but as yet no study has been carried out on the distribution both of virulent and non-virulent strains of *Pseudomonas tumefaciens* in soils which have been growing different crops, as, for example, virgin soils, nursery soil, pasture, and corn-land. The results of such a study, together with data on the overwintering of the pathogene in sterilized and unsterilized soils of various types, are reported in this paper.

Before discussing the results obtained, the literature bearing directly on the subject will be briefly summarized.

LITERATURE REVIEW

Fulton (2), working with the citrus canker organism, *Pseudomonas citri* Hasse, found that the number of pathogenic organisms in artificially infested soil decreased with time. The pathogene had disappeared after two weeks had elapsed. In order to prove the presence or absence of the organism in question, he inoculated mature leaves of grapefruit with soil suspensions.

[1] The writer wishes to express his gratitude to Drs. I. E. Melhus and J. H. Muncie for valuable criticisms and suggestions made from time to time during the progress of this work. These studies have been carried out in connection with the crown gall project in which the Crop Protection Institute of the National Research Council; United States Department of Agriculture, Office of Mycology and Disease Survey; Iowa State College; and University of Wisconsin are cooperating.

In the case of potato black-leg, caused by *Bacillus atrosepticus* van Hall, Rosenbaum and Ramsey (11) were unable to reisolate the organism from the soil of their wintering-over test plots. Kotila and Coons (4), while able to prove the presence of *B. atrosepticus* in the soil immediately surrounding diseased plants by inoculating potato slices directly with soil particles, found that the organism either quickly disappeared from the soil or lost its pathogenicity.

Goldsworthy (3), working with the longevity of the cauliflower spot disease organism, *Pseudomonas maculicolum* McCulloch, in the soil, attempted to recognize the presence of the pathogene on poured plates by means of agglutination phenomena. He concluded that typical colonies, which he isolated from such plates, that gave the same agglutination titre as the pathogene in question were identical species. This conclusion is open to the criticism that no inoculation experiments are reported with these organisms and, as Durham (1), Stevens(13), and Wilson (14) have shown, that different varieties and even species may agglutinate at the same titre. More conclusive results would doubtless be reached by a combination of agglutination and inoculation.

In 1922, Riker (10) showed that the crown gall organism retained its viability for a year in sterilized soil. Reddick and Stewart (9), also using sterilized soil, were able to isolate *Pseudomonas tumefaciens* from clay after 43 days, from loam and quartz sand after 186 days. They failed, however, to obtain the organism after 298 days. The data from similar experiments on unsterilized soils were unfortunately lost. In addition to these data on longevity, they showed, that, when free from competition, *Ps. tumefaciens* could withstand low temperatues and repeated changes of temperature at or near the freezing point in the soil and that it might move considerable distances with the currents of soil water. Their criterion of viability was successful inoculation of tomato plants by means of soil suspensions.

While all these workers studied the longevity of the crown gall organism in sterile soil, Muncie (5), in one experiment, recorded the presence of the viable pathogene in unsterilized soil after 102 days, and later (6), in a second trial, obtained successful inoculations with a suspension from an unsterilized loam which had been infested 154 days previously. More recently the writer (8) successfully isolated *Ps. tumefaciens* in a virulent state from unsterilized soils 10 months after their infestation. Since this report these studies have been carried further, and the results are presented in this paper.

MATERIALS AND METHODS

Soils

The experiments reported in this paper were carried out on three types of soil in the laboratory: clay, loam, and sand. The clay was a sub-soil clay

collected from a building excavation at Ames, Iowa. The loam was a greenhouse soil, high in organic matter. The sand was quartz sand.

In addition to the laboratory trials, soils in two different fields of the silt loam type were artificially infested. In the first field the infestation was produced by means of pure culture, and in the second field by infected plant materials.

Each of the three soils used in the laboratory was divided into two parts and placed in flasks of 300 cc. capacity. One portion was sterilized in the autoclave at 20 pounds for 6 hours. Sterilization was tested by means of poured plates using potato dextrose agar as a medium. No colonies were found on these plates after 6 days. Both portions were then infested by introducing into the flasks enough of a 72-hour-old broth culture of *Ps. tumefaciens* to wet the samples thoroughly. The flasks were stored in an ice-box at 12–14° C. The moisture content was maintained by the addition of sterile distilled water to the flasks as need arose.

For the overwintering studies, similar soils, that is, clay, loam, and sand, sterilized and unsterilized, were artificially infested with a 72-hour-old broth culture of *Ps. tumefaciens* after they had been tubed. Fifteen tubes were filled to a depth of 3 inches with each kind of soil, infested, and buried at a depth of 6 inches on October 5, 1926. These tubes remained in the ground all winter. On March 10, 1927, they were dug up, and isolations were made from their contents.

Besides the soil cultures that were buried in the ground, 10 tubes of a sterilized greenhouse loam and 10 tubes of unsterilized greenhouse loam were artificially infested with a 72-hour-old broth culture of the crown gall organism and placed in two pots on the surface of the ground outside the greenhouse. The tubes were covered with soil in the pot, but the pots were left uncovered. These pots were placed outdoors on December 10, 1926. Tubes were removed at intervals up to March 10, 1927, when the trial was finished. The tubes were about half filled with soil.

Finally two soil samples furnished by Muncie (6) were added to the series of soils mentioned above. These samples consisted of clay and greenhouse loam, sterilized and unsterilized, infested on January 13, 1926. These samples were carried in the laboratory in the same manner as the flasks, and isolations were made at irregular intervals.

Isolations

Since the object of the isolations was to secure a specific organism, *Ps. tumefaciens*, and not, as with the usual soil bacteriologic technic, the total number of organisms present, a special method was developed which took advantage of certain peculiarities of the nutritive requirements of this or-

ganism. With the larger samples, approximately 20 grams of the soil sample were dropped into a bottle containing 100 cc. of sterile distilled water and shaken for 2–5 minutes. The bottle was then allowed to stand for from 3 to 8 hours with occasional shaking. After a very vigorous final shaking the bottle was rested for 10 minutes to allow the heavy particles of soil to settle out. From the supernatant, but still cloudy, liquid, dilutions of 1–1000, 1–10,000 and 1–100,000 were made, using sterile pipettes and sterile distilled water, and the usual other precautions to maintain aseptic conditions during transfer. Ordinarily three plates were poured from each dilution, using crystal violet bile agar as devised and already described by the author (7), as follows: 1 cc. of the dilution to be poured was added to a flask containing 25 cc. of crystal violet bile agar, and, after thorough mixing, the contents were poured into three sterile petri dishes using approximately one-third of the mixture for each dish. After the agar had solidified the plates were inverted and incubated at room temperature (*circa* 25° C.). After 48 hours the plates were examined daily with a hand lens for 10 days or more. Usually the colonies of *Ps. tumefaciens* came up in 48–72 hours in this medium.

As a check on the presence of the crown gall organism in the dilutions, three healthy young tomato plants were inoculated with each dilution of soil by dipping a sterile needle into the soil suspension and then scratching the upper portion of the stem of the plants in from 5 to 10 places.

Later, when colonies resembling *Ps. tumefaciens* appeared on the plates, they were marked and inoculated into three tomato plants by puncturing the tender portion of the top. The plants were cared for in the greenhouse under conditions suitable for continued, rapid, succulent growth. Trial plants were examined for galls at intervals during three to six weeks and were then discarded. Care was taken to prevent accidental infection from soil splashing or by other means, by separating the plants from one another by 3 inches in all directions.

Crystal violet bile agar was used as the medium for the isolations because, as reported in a previous paper (7), this medium eliminates gram positive bacteria and most of the coccus forms and checks the growth of the molds. The use of potato dextrose agar which was used in the earlier isolation studies was discontinued since *Ps. tumefaciens* grows more slowly on this medium than on the crystal violet bile agar, and the various contaminants—spreaders, molds, etc.—grew on it so well that the plates soon became overrun with these forms, making close examination impossible.

LONGEVITY OF PSEUDOMONAS TUMEFACIENS IN SOILS IN THE LABORATORY

The longevity of *Pseudomonas tumefaciens* in the laboratory was tested in sterilized and non-sterilized clay, loam, and quartz sand. The results of these experiments are presented in table 1.

TABLE 1.—*Longevity of Pseudomonas tumefaciens Sm. and Town. in sterilized and non-sterilized soils at room temperature in the laboratory as determined by isolation and inoculation of the organisms into tomato plants*

Date of infestation	Date of isolation	No. days organisms in soil	No. plants inoculated from each sample	No. plants infected with colonies from						No. controls infected
				Sterilized			Unsterilized			
				clay infested	loam infested	sand infested	clay infested	loam infested	sand infested	
June 3, 1926	Aug. 31, 1926	90	3	3	3	3	3	3	3	0
do	Oct. 2, 1926	122	3	3	3	3	3	3	3	0
do	Nov. 10, 1926	160	3	3	3	3	0	3	3	0
do	Mar. 1, 1927	271	3	3	3	3	2	3	2	0
do	Mar. 25, 1927	295	3	3	3	3	0	2	2	0
do	April 10, 1927	311	3	3	3	3	0	3	3	0
do	May 17, 1927	349	6	6	6	6	1	3	5	0
do	June 8, 1927	371	6	6	6	6	0	1	3	0
do	July 26, 1927	420	6	6	6	6	1	1	2	0
do	Aug. 31, 1926	231	3	3	...	...	...	0	...	0
[a]Jan. 13, 1926	Oct. 2, 1926	263	3	0	...	...	...	0	...	0
do	Nov. 2, 1926	294	3	0	...	...	...	3	...	0
do	Nov. 10, 1926	302	3	0	...	...	...	3	...	0
do	Mar. 14, 1927	424	5	0	...	...	...	1	...	0
do	May 17, 1927	491	6	0	...	...	...	0	...	0

[a] These soil samples were kindly supplied by Dr. J. H. Muncie and were used in his studies (6, p. 83, Table V).

From these data it will be seen that, under the conditions of these trials, *Ps. tumefaciens* was still alive and active for many days after the soil was infested. In the series infested on June 3, 1926, the organism was still viable after 420 days in all three types of soil which had been sterilized before infestation. In the case of the soils which were not sterilized the data show that the pathogene decreased in numbers in the clay much more rapidly than in the loam or sand, although one successful infection was obtained from six inoculations made after 420 days. In the case of the loam and sand, inoculations made after 420 days were also successful, although there is some evidence of attenuation in that the number of infections is reduced as the time between infestation and inoculation increases.

The two soil cultures infested and reported on by Muncie (6) were continued, and after 231 days the organism was found in the sterilized clay. Unfortunately this soil sample became contaminated by a mold, and no inoculations produced infection thereafter. With the other culture of unsterilized loam, tomatoes became infected when inoculated after 424 days, although the gall produced in the last trial was very small. An isolation from this gall yielded a pure culture of the pathogene, which in turn produced typical crown gall on inoculated tomato plants.

These experiments show that *Ps. tumefaciens* may live for a year at least in both sterile and unsterilized soils in the laboratory. It also seems clear that it will live longer in sterile soil, where it is not in competition with other organisms, and that it may live longer in sandy soils than in clays. That the pathogene becomes attentuated in the unsterilized clay, loam, and sand is shown by the gradual reduction in the number of successful inoculations from these soils. No data were obtained by counting the number of organisms in the soil, since this method was regarded as too unreliable due to the difficulty of recognizing the crown gall organism with sufficient accuracy.

When one considers the fact that each different lot of soil was infested with an approximately equal number of crown gall bacteria in an equal volume of medium, it would seem that competition existing between the micro-organisms in the soil has a greater influence upon the longevity of *Ps. tumefaciens* than the apparent nutritive content of the soil medium. This may account for the gradual decrease in the number of infections obtained on the tomatoes inoculated from suspensions of infested clay as compared with sand, since clay normally harbors greater numbers of micro-organisms.

OVERWINTERING OF PSEUDOMONAS TUMEFACIENS IN THE OPEN

It has been shown above that the crown gall organism can live in the soil under laboratory conditions for more than a year. However, these

conditions did not subject the organism to so severe a test as would occur in the field. Therefore samples of sterilized and unsterilized soil were infested with *Ps. tumefaciens* and exposed to outside conditions as already described. These samples were either free in the field or confined in glass containers. Isolations were made at various times from those soil samples kept on the surface of the soil, and the organisms isolated were inoculated into healthy tomato plants. Inoculations were also made directly from the soil suspensions, and infection was obtained on tomatoes inoculated from each sample. Three control plants were held for each inoculation. The results of these trials are given in table 2.

Fom these data it is clear that *Ps. tumefaciens* can live over winter in the soil, since all five trials with both soils yielded the pathogene after 5, 18, 26, 76, and 90 days. The temperature during this period ranged from – 23° C. on December 15, the lowest temperature noted during this time, to 15° C. on January 2, 1927.

The temperature records from October 5, 1926, to March 10, 1927, were obtained from the Weather Bureau station of the United States Department of Agriculture at Ames, Iowa.

Except for three days, the minimum daily temperatures in the month of October, 1926, were above freezing. These ranged from 15° C. to – 5° C., the maximum daily temperatures for the same period ranging from 25° C.

TABLE 2.—*The overwintering of Pseudomonas tumefaciens Sm. and Town., out of doors, as determined by inoculations with organisms isolated from unsterilized and sterilized soils infested Dec. 10, 1926*

Kind of soil	No. of samples	Date of isolation	No. days outdoors	No. tomatoes inoculated	No. tomatoes infected	No. controls infected
Unsterilized						
greenhouse loam	1	Dec. 10, 1926	0	3	3	0
do	1	Dec. 15, 1926	5	3	3	0
do	1	Dec. 28, 1926	18	3	3	0
do	1	Jan. 5, 1927	26	3	3	0
do	1	Feb. 24, 1927	76	3	3	0
do	5	Mar. 10, 1927	90	6	6	0
Sterilized						
greenhouse loam	1	Dec. 10, 1926	0	3	3	0
do	1	Dec. 15, 1926	5	3	3	0
do	1	Dec. 28, 1926	18	3	3	0
do	1	Jan. 5, 1927	26	3	3	0
do	1	Feb. 25, 1927	76	3	3	0
do	5	Mar. 10, 1927	90	3	3	0

to 4° C. In November, the minimum temperatures were at or below freezing, except for 5 days. These ranged from 9° C. to – 12° C. The maximum temperature for the same period ranged from 18° C. to – 5° C., being below zero for 5 days at random.

The minimum daily temperatures in the month of December were below freezing, ranging from 0° C. to – 22° C. The maximum temperatures for the same period were at, or slightly below, freezing except for 14 days in which they ranged from 13° C. to – 13° C. The minimum temperatures in January, 1927, were below freezing, ranging from 2° C. to – 23° C., except for 2 days when it registered 2° C. and 1° C. The maximum temperatures during this month were above freezing except for 12 days. These ranged from 10° C. to – 13° C. During this month the minimum daily temperature reached the lowest point for the entire period of the experiment. The maximum temperatures in February were above freezing—14° C. to – 7° C.—except for 4 days. The minimum temperatures for the same period were below freezing except for 3 days, ranging from – 18° C. to 2° C. This month marked the rise in temperature which continued up to March. During March the maximum temperatures were above freezing except for 1 day. The minimum temperatures for the first 10 days of the month ranged from – 10° C. to 3° C.

There was, during the course of the experiment, no period of more than 6 days in which the maximum daily temperatures remained continuously

TABLE 3.—*Overwintering of Pseudomonas tumefaciens Sm. and Town., as determined by inoculations with organisms isolated from sterilized and non-sterilized soils buried 6 inches in the soil out of doors on Oct. 5, 1926*

Kind of soil	No. of samples	No. days outdoors	No. tomatoes inoculated	No. tomatoes infected from soil suspensions	No. tomatoes infected from plated colonies	No. controls infected
Sterilized sand	13	156	17	13	4	0
Sterilized clay	12	156	15	9	5	0
Sterilized loam	12	156	17	5	11	0
Non-sterilized sand	15	156	22	10	8	0
Non-sterilized clay	12	156	17	11	6	0
Non-sterilized loam	12	156	18	9	9	0

below the freezing point. During the months of December and January the temperature reached the lowest maximum and minimum marks. The fluctuations during these months were numerous and abrupt.

In the case of the buried samples, isolations were made 156 days after they were placed underground. The results of these isolations and inoculations into tomato plants are shown in table 3. It will be noted that in all cases the pathogene was reisolated from the soils and successfully reinoculated into tomato plants. In a few cases the first galls produced from the reisolation cultures were very small. In such instances the organism was reisolated from the gall and used in inoculating other tomato plants. Normal-sized galls resulted in all these inoculations. Recovery of the pathogene was somewhat more difficult from the tubes of sterilized soil which were plugged with cotton and buried outside, because the cultures were contaminated when the plugs became soaked with soil water.

Further to substantiate the possibility of the overwintering of the crown gall pathogene, soil in the field was infested with the bacterium both from pure cultures in broth and from finely chopped tomato galls ploughed into the soil on October 5, 1926. The reisolations and inoculations from the soils of these field plots were made on March 10, 1927, after an elapse of 156 days. In this instance successful inoculations from the soil suspensions were made only in the case in which pure cultures had been used for the source of infestation. However, virulent colonies of *Ps. tumefaciens* were isolated from soil subjected to both types of infestation. Smith, Brown, and Townsend (12) reported the successful renewal of growth of *Ps. tumefaciens* in pure culture after it was subjected to a temperature of from 0° C. to −14° C. for two weeks.

In another experiment, 4 castor bean plants outside the greenhouse were inoculated with *Ps. tumefaciens* at internodes some time during the early fall. The galls had grown to a size of about 2 cm. before winter set in and the plants were killed. From time to time during the winter months isolations were made from the galls on the dead host and in no case was the pathogene recovered. It is possible that the crown gall pathogene does not live over winter on a dead host under Iowa conditions.

From these experiments it is clear that *Ps. tumefaciens* can overwinter readily in the soil in the absence of its host.

DISTRIBUTION OF PSEUDOMONAS TUMEFACIENS IN SOILS BEARING VARIOUS CROPS

In addition to the studies on longevity and overwintering of *Ps. tumefaciens* in the soil, a survey was made of soils from 12 nurseries representing 9 states to determine, if possible, the distribution of the crown gall organism in these soils, and the relation of its presence to the crop being grown on the soil.

The samples of soil from 12 different nurseries in 9 states were collected by Dr. Muncie and Messrs. Layton, Shippy, and Johnston, while on crown gall survey in the field. They took care that the samples were kept separate. As soon as they reached the laboratory, the soils were placed in pots in the greenhouse and kept moist. Isolations were made in the same manner as in the longevity studies, and inoculations were made from soil suspensions and from colonies suspected of being *Ps. tumefaciens*.

The description of the soils used and the results of the isolations and inoculations are tabulated in table 4.

TABLE 4.—*Distribution of Pseudomonas tumefaciens in various field soils*

Culture no.	No. of samples	No. of trials	Present crop	Past crop	Crowngall observations	Presence of organisms resembling the pathogene in plates[a]	Infection on tomatoes from colonies and soil suspension[b]
A–1	1	2	Farm crops	Farm crops	Absent	–	–
A–2	1	2	Pear	Pear	Abundant	+	–
B–1	1	–	Apple	Farm crops	Slight	0	0
B–2	1	1	do	do	do	+	–
B–3	1	1	Evergreen	Evergreen	Absent	–	–
B–4	1	1	Rose	Rose	Badly galled	+	–
C–1	1	5	Evergreen	Evergreen	Absent	–	–
C–2	1	3	Apple	Ornamentals	Slight	+	–
C–3	1	3	Cherry	do	do	+2	–
C–4	1	3	Apple	do	do	+3	+2
C–5	1	3	do	do	13 per cent	+3	+2
D–1	1	1	do	do	Badly galled	–	–
D–2	1	1	do	do	Slight	+	–
D–3	1	1	Evergreen	Evergreen	Absent	+	–
D–4	1	1	Rose	Rose	Badly galled	+	–
E–1	1	2	Poplar	Poplar	Absent	–	–
E–2	1	1	Peach	Peach	Slight	–	–
F–1	1	1	Apple	Old orchard	do	+	–
F–2	1	1	do	Nursery stock	do	–	–
F–3	1	1	do	do	do	–	–
F–4	1	1	do	Pasture	Absent	–	–
F–5	1	1	do	Apple	Badly galled	+	–
F–6	1	1	Peach	Nursery stock	Absent	+	–
F–7	1	1	Apple	do	do	–	–
F–8	1	1	do	Pasture	Some	+	–
G–1	1	1	Peach	Nursery stock	Badly galled	–	–
G–2	1	1	do	do	do	–	–
G–3	1	2	do	do	Clean	–	–
G–4	1	3	do	do	10 per cent	+2	+2
H–1	1	1	Apple	do	Moderate	–	–
H–2	1	1	do	do	do	–	–
H–3	1	1	do	do	do	+	+
H–4	1	1	do	do	do	+	+
H–5	1	1	do	do	do	+	–
H–6	1	1	do	do	do	+2	+2
H–7	1	1	do	do	do	+	–

TABLE 4.—(*Continued*)

Culture no.	No. of samples	No. of trials	Present crop	Past crop	Crowngall observations	Presence of organisme resembling the pathogene in plates[a]	Infection on tomatoes from colonies and soil suspension[b]
H–8	1	1	Apple	Nursery stock	Moderate	+	–
H–9	1	1	do	do	do	+	–
H–10	1	1	do	do	do	+	+
H–11	1	1	do	do	do	+	–
H–12	1	1	do	do	do	+	–
H–13	1	1	do	do	do	+	–
I–1	1	1	do	do	Slight	–	–
I–2	1	1	do	do	do	–	–
I–3	1	1	do	do	Clean	–	–
I–4	1	1	do	do	do	–	–
I–5	1	1	Peach	Ornamentals	Slight	–	–
J–1	1	1	Corn	Apple	Badly galled	+	–
J–2	1	1	Apple	do	Slight	+	–
K	9	9	Peach	Peach	Clean	+2	–
L	6	6	Apple	Apple	Absent	+2	–
M–1	1	1	Peach	No nursery stock	do	+	–
M–2	1	1	do	do	do	+	–
N–1	13	13	Apple	Apple	Clean	+3	–
N–2	4	4	do	do	Slight	+4	–
O–1	1	1	do	do	do	–	–
O–2	1	1	Evergreen	Evergreen	Absent	–	–
P–1	3	3	Corn	Corn	do	–	–
P–2	3	3	None	None	do	–	–
P–3	3	3	do	do	do	–	–
P–4	3	3	Apple	Apple	do	+1	–

[a] – denotes the absence and + denotes the presence of organisms resembling *Ps. tumefaciens*. A number accompanying the sign denotes the number of trials made.

[b] Infections from colonies and soil suspensions were identical. No infection is indicated by – and infection by +. A number accompanying the sign denotes the number of trials made; 0 denotes no inoculation.

With the exception of samples F8 and K, which were of a sandy nature, the soil type in the nurseries was a heavy loam. In all cases except K, L, and M, the soil had been fertilized by turning under a green manure crop not more than three years before the samples were taken.

These data show that the organisms resembling *Ps. tumefaciens* are not generally found in soils from which crops susceptible to this pathogene are absent. From only 1 of 14 samples was an organism of this type isolated. Tomato plants inoculated with this organism did not become infected.

On the other hand, organisms resembling *Ps. tumefaciens* were isolated 41 times from 96 soil samples; and of the 41 cultures, 7 proved pathogenic for tomato upon inoculation.

The data also indicate that the pathogenic form of *Ps. tumefaciens* is localized in the field, because infection was obtained after inoculation with soil suspensions only in cases in which the samples taken had been in close proximity to true galls. Soil suspensions from samples taken at a distance from the crown-galled trees did not infect tomatoes upon inoculation.

In order to eliminate the possibility of the occurrence of organisms resembling *Pseudomonas tumefaciens* as contaminants in air, water, or common disinfectants used in the experiments, four trials were made to detect the presence of such organisms in these materials. Potato dextrose plates were poured and exposed to the air from 5 to 20 minutes outdoors and in the laboratory. The tap water and disinfectants were treated as soil suspensions. Organisms resembling *Ps. tumefaciens* were found in no case.

SUMMARY

Pure virulent cultures of *Ps. tumefaciens* were recovered from infested sterilized clay, loam, and quartz sand after 420 days. The infested unsterilized samples of clay, loam, and quartz sand also yielded the pathogene after 420 days, but the number of pathogenic colonies in these samples decreased in the order of clay, loam, and sand. Infection was produced from another sample of infested unsterilized clay after 424 days.

In the overwintering studies *Ps. tumefaciens* was recovered from artificially infested soils after being subjected for 90 days to out-of-door temperatures ranging from −23° C. to 15° C. From infested samples buried at a depth of about 6 inches in the soil the organism was recovered after 156 days of winter temperatures. In the field in soils infested with pure cultures and in others infested with diseased tissue, the organism was recovered in both cases after passing the winter in the soil in the absence of the living host.

Organisms resembling *Ps. tumefaciens* were recovered in 41 cases from 96 nursery soil samples; and of these, 7 proved pathogenic for tomato upon inoculation. In each case the pathogene was recovered from soils upon which hosts susceptible to *Ps. tumefaciens* were growing. Suspensions of 14 samples of soil on which non-susceptible crops were grown for a number of years did not infect tomatoes. No colonies resembling *Ps. tumefaciens* were obtained from the air, tap water, or common disinfectants.

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PHYSIOLOGIC SPECIALIZATION IN PUCCINIA SORGHI[1]

E. C. Stakman, J. J. Christensen, and H. E. Brewbaker

INTRODUCTION

In recent years considerable work has been done in developing disease-resistant lines of corn. But relatively little attention has been paid to corn rust, caused by *Puccinia sorghi* Schw., because it has caused so little injury that it is generally considered of only minor economic importance. However, Stakman and Christensen (9) pointed out that the rust might be very destructive to certain selfed lines of corn and should be taken into consideration in any program of corn breeding. For this reason the writers obtained data on the relative resistance to rust of a large number of selfed lines of different varieties of corn and made attempts to ascertain whether there was any correlation between the reaction of the lines to rust and smut. In addition, there was some field evidence that there might be physiologic forms of *P. sorghi*, and a study therefore was made to find out whether there actually were such forms.

FIELD STUDIES ON THE RELATIVE RESISTANCE OF SELFED LINES

In 1923 there were striking differences in the rust reaction of selfed lines of Squaw Flint grown in the plant breeding nursery at University Farm, St. Paul, Minnesota. In 1924 rust inoculum was apparently not so abundant as in the previous year, although one selfed strain of Northwestern Dent was rather heavily infected. In 1925 a few selfed strains of Squaw Flint again were heavily infected. The leaves, husks, necks, and tassels of these strains were so severely affected that the plants died prematurely. There was considerable variation in the reaction of other selfed lines of Squaw Flint and of certain inbred lines of other varieties. (See figures 1 and 2.)

As rust seldom seemed to be destructive to ordinary varieties of corn grown in the field, the severity of attack on some of the selfed lines sug-

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The writers are indebted to W. Butler, J. H. Craigie, and H. A. Rodenhiser for collections of rusts.

The work was done as one phase of a project on the development of disease-resistant varieties of farm crops, carried on cooperatively by the Sections of Plant Genetics and Plant Pathology. The field observations were made in the regular corn breeding plots of the Section of Plant Genetics.

gested the necessity of taking into consideration susceptibility to rust in selecting the better lines.

Conclusions regarding varietal resistance of corn to diseases are not generally considered very reliable because corn usually is very heterozygous. But Weber (12), in 1921, noted differences in the susceptibility of the several species of corn to rust. *Zea everta* (pop-corn) was the most resistant, while *Z. saccharata* (sweet corn) seemed to be the most susceptible. Weber also tested six varieties of dent corn but found only slight differences in their reaction to rust. Furthermore, field observations made over a period of years in Minnesota indicated that there were marked differences in varietal susceptibility to rust, both of field corn and sweet corn. Of 22 varieties of sweet corn tested, Golden Bantam was by far the most susceptible, while Crosby and Country Gentleman were quite resistant. Squaw Flint was the most susceptible variety of field corn under observation. In general, most of the varieties of field corn grown in Minnesota appear to be sufficiently resistant to remain uninjured in the field.

In 1924, Mains et al. (8) and Holbert et al. (4) called attention to striking differences in the susceptibility of different selfed lines of corn. Stakman and Christensen (9), in 1926, also pointed out that there were

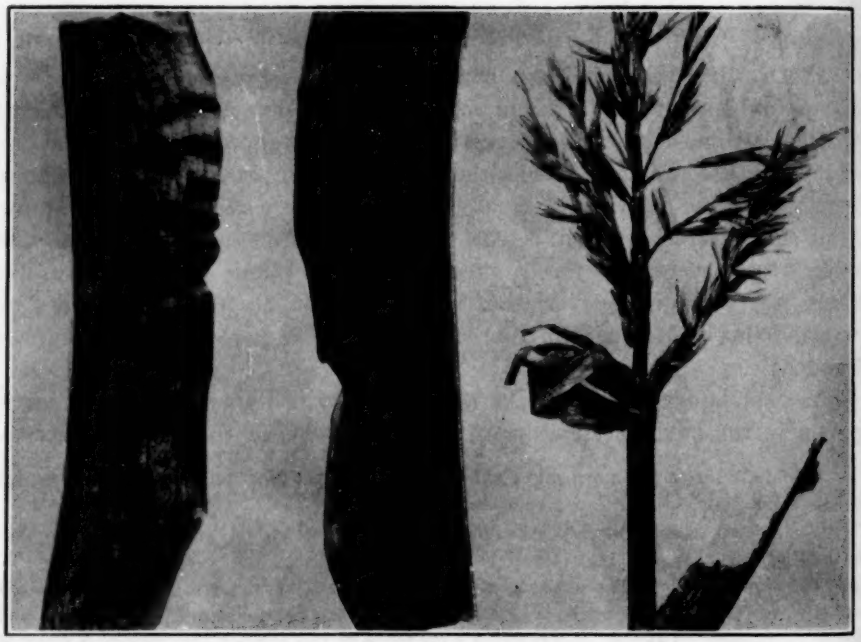

FIG. 1. Minn. No. 13, culture 86, selfed one year. Severely attacked by corn rust. A. Lower side of leaf. B. Upper side of leaf. C. Tassel infection.

great differences in the susceptibility of different selfed lines. It seemed desirable, therefore, to obtain as much information as possible regarding the relative susceptibility of the selfed lines which were grown in the plant breeding plots at University Farm.

The lines of corn studied during the summer of 1925 had been selfed from 2 to 7 years, most of them from 4 to 5. About 40 plants of each line were grown. They were planted 1 foot apart in rows 3½ feet apart. Observations were made on the degree of natural infection of rust and smut on 171 of these lines. The data are summarized in table 1.

TABLE 1.—*The reaction of 171 selfed lines of corn to rust and smut in the plant breeding plots at University Farm, St. Paul, Minnesota, 1925*

Variety	No. lines tested	Years selfed	Infection classes and number of lines in each class					
			Rust			Smut		
			Resistant	Intermediate	Susceptible	Resistant	Intermediate	Susceptible
Minn. No. 13	17	5[a]	6	9	2	7	8	2
Longfellow	18	2 to 6	5	12	1	5	8	5
Northwestern Dent	18	5 to 7	10	8	0	9	7	2
Squaw	46	2 to 7	3	23	20	13	17	16
Rustler	18	5 to 7	13	5	0	8	8	2
King Phillip	39	3 to 6	11	28	0	8	8	23
Minn. No. 23	15	3	12	3	0	0	9	6
Total	171		60	88	23	50	65	56

[a] Except two lines which were selfed 1 and 2 years respectively.

There were marked differences in resistance and susceptibility, both to rust and smut. It is well known that there is great variation in smut reaction of selfed lines obtained from a commercial variety, and the need of selecting for smut resistance is well recognized (2, 3, 5, 6).

Reaction to rust within inbred lines has not been studied so extensively as that to smut. From observations made in the plant breeding nursery since 1923 it appears that susceptible lines of Squaw are relatively numerous but that highly susceptible inbred lines of other varieties are rather uncommon. Susceptibility or resistance to smut appeared to be entirely independent of the reaction to rust, and many lines which appeared resistant to both diseases were found (table 2).

There were many different types of rust reaction. On some of the lines there were relatively few, but very large, pustules; on other lines there were numerous small pustules; on others there were large pustules sur-

FIG. 2. Two selfed lines of corn, showing difference in susceptibility to rust under field conditions, 1925. A. (Culture 1078) 7 years selfed, susceptible.
B. (Culture 1054) 3 years selfed, resistant.

TABLE 2.—*Relation between degree of rust infection and degree of smut infection in the 171 selfed lines of corn listed in table 1*

		Reaction to rust			
		Resistant	Intermediate	Susceptible	Total
Reaction to smut	Resistant	18	23	9	50
	Intermediate	26	30	9	65
	Susceptible	16	35	5	56
Total		60	88	23	171

rounded by distinct necrotic areas; and on still other lines there were both large and small pustules, either with or without chlorosis or necrosis. This suggested the presence of two or more physiologic forms of *P. sorghi*.

PHYSIOLOGIC SPECIALIZATION WITHIN PUCCINIA SORGHI

In 1926 Stakman and Christensen (9) published a preliminary note on physiologic specialization in *Puccinia sorghi* and *Ustilago zeae*; and Mains (7) also showed that there are forms of *P. sorghi*. The experiments recorded in the present paper are a continuation of work begun in 1924, when two forms were isolated, one of which infected teosinte heavily, while the other infected it weakly.

Materials and Methods

Numerous collections of *P. sorghi* were obtained from the Mississippi Valley, and, in addition, one was obtained from New Hampshire and one from Winnipeg, Canada. About 45 inbred lines of corn and one or two lines of teosinte were inoculated with most of these collections.[2] As a result of the preliminary inoculations, 8 selfed lines of corn were selected as differential hosts.

The methods used in making inoculations were essentially the same as those described by Stakman and Piemeisel (10) in inoculating cereals and grasses with *Puccinia graminis*.

The method of indicating the degree of infection was adapted somewhat from that used by Stakman and Levine (11) in their work with *P. graminis tritici*. However, the types of infection produced by *P. sorghi* were neither as distinct nor as consistent as those caused by *P. graminis*. Therefore it was necessary to modify their system somewhat. In table 3 the numbers are used to indicate the size of pustules as follows:

[2] Most of the seed of the selfed lines of corn used in the greenhouse experiments was furnished by the Section of Plant Genetics, University of Minnesota. Some was obtained from Dr. R. J. Garber, West Virginia Agr. Exp. Sta. Seed of teosinte was furnished by Mr. F. D. Richey, U. S. Department of Agriculture.

1—Uredinia very small, rather scattered, and not confluent.

2—Uredinia somewhat larger than in 1, usually scattered, and not confluent, except in rare cases.

3—Uredinia moderately large, seldom confluent.

4—Uredinia large and frequently confluent.

Plus or minus signs after the figure indicating the size of pustules means plus or minus fluctuation within the class. For example, 1 – means that the pustules are extremely small, even for class 1.

The letter n following the figure indicates the presence of necrotic areas.

Plus and minus signs after the n indicate the degree of necrosis. For instance, n indicates moderate necrosis, n – rather indistinct necrosis, and n + very decided necrosis. There appear to be practically all combinations of pustule size and necrosis.

Results

It is evident that there are at least seven distinct physiologic forms which can be recognized readily by their effect on eight selfed lines of corn (table 3). The differences in the infection capabilities are shown more clearly in the following analytical key:

ANALYTICAL KEY FOR IDENTIFICATION OF PHYSIOLOGIC FORMS OF *Puccinia sorghi*

Golden Bantam, culture 1–S, very resistant	Form 1
Golden Bantam, culture 1–S, very susceptible	
Longfellow, culture 611–A, very resistant	
Minnesota No. 13, culture 213, very resistant	Form 2
Minnesota No. 13, culture 213, very susceptible	
Rustler, culture 396, resistant	Form 3
Rustler, culture 396, susceptible	Form 4
Longfellow, culture 611–A, susceptible	
Minnesota No. 13, culture 213, resistant	Form 5
Minnesota No. 13, culture 213, susceptible	
Northwestern Dent, culture 507, resistant	Form 6
Northwestern Dent, culture 507, susceptible	Form 7

Form 1, isolated from a collection obtained from San Antonio, Texas, was by far the weakest in pathogenicity. In fact, it was so weak that it was rather difficult to keep it in culture. This form did not develop normally even on culture 1–S, a strain of Golden Bantam, which was very susceptible to 17 of the rust cultures obtained. At least 22 lines of corn were inoculated with form 1, and not a single one was susceptible. As a rule it is relatively easy to maintain and propagate corn rust in the greenhouse, but form 1 was lost before a congenial host could be discovered. In addition to differences in pathogenicity, form 1 also differed in appearance from the other six forms. The color of the uredinia was much lighter than that

TABLE 3.—*Reaction of eight selfed lines of corn to seven physiologic forms of corn rust.*

Form no.	Place of collection	Lines of corn and rust reaction								
		Golden Bantam, culture 1–S	Eldridge, culture 3–5–2–2–1–1	Minn. No. 13, culture 212	Minn. No. 13, culture 213–A	Rustler, culture 396	Rustler, culture 410	Northwestern Dent, culture 507	Longfellow, culture 611–A
1	San Antonio, Texas	1 – n +	.....	.....	0n + to 1n –	.....	.....	.....	3 =
2	Stillwater, Oklahoma	4 + n –	0n + to 1n	2 to 3n –	1n +	3 + n	2 + n	3n	1 + n +
3	Concordia, Kansas	4 – n	0n + to 1n	1n + to 2 + n	3 =	2 + n	2n –	3	0n + to 1n +
4	University Farm, Minnesota	4 – n –	.....	1n + to 2 + n	3 +	4n +	2n –	3 +	0n + to 1n +
5	Lincoln, Nebraska	4 + n –	0n +	3 + n	1n +	4 – n	4 –	3n –	3 = n
6	Iowa	4n –	.....	2n –	3 + n	3 ± n	3 –	2 –	3n
7	Hutchinson, Minn.	4n	0n + to 3n	3 +	3n	1n + to 3n	3 –	3 +	3n

of the other forms, and under the microscope the spores also were much lighter in color than those of the other forms. It is barely possible that this difference in color may have been due to the fact that the uredinia of form 1 never were produced on a congenial host, but this seems unlikely. Form 1 also differed from all of the others in the fact that it never produced any teliospores, although other forms in neighboring booths in the greenhouse produced them in abundance on certain hosts. Wellensiek (13) observed that the formation of teliospores often was induced by the resistance of the host, but this was not true of form 1.

There may be a number of physiologic forms in the same general locality. Two forms, numbers 4 and 7, were isolated from material collected in Minnesota, and there is some evidence that there is a third form in the State. Forms 4 and 7 were obtained from corn grown on University Farm, St. Paul; while form 7 was obtained also from field corn near Hutchinson, Minn., about 60 miles west of St. Paul, and from three other localities in Minnesota, as well as Henniker, New Hampshire. It seems quite likely, therefore, that it may be widely distributed.

It is quite likely that there are numerous other physiological forms which could be recognized readily if more collections were made and more differential hosts inoculated. Because of the fact that selfed lines of corn may be heterozygous for rust reaction, it would be rather difficult to place the identification of forms of *P. sorghi* on as definite a basis as that of some of the cereal rusts.

DISCUSSION AND CONCLUSIONS

By far the best method of reducing losses from certain corn diseases is the development of disease-resistant lines. However, it already has been shown (1) that there are numerous physiologic forms of the corn smut fungus, *Ustilago zeae*. It has been shown in this paper, as well as by the work of Mains, that there also are physiologic forms of *P. sorghi*. It is imperative, therefore, to take these facts into consideration in any program for the development of smut- and rust-resistant lines of corn.

The facts brought out in this paper show clearly that a pathogene such as *P. sorghi*, which is relatively innocuous to most varieties of corn now grown commonly, may be, under certain conditions, very destructive to selfed lines which are produced for the purpose of general varietal improvement or for resistance to certain diseases. It is important to realize that in producing selfed lines of corn one is in reality producing "physiologic forms" of a host plant and that these forms may be extremely susceptible to a pathogene hitherto considered of little economic importance.

It requires several years of continuous self-fertilization to develop inbred lines of corn which are relatively homozygous. It is the common practice

of corn breeders during this period of continuous inbreeding to select rigidly for freedom from striking abnormalities and, in some cases, susceptibility to various diseases. Strains which are highly resistant to at least some physiologic forms of *U. zeae* have been obtained as a result of such selections. Rust appears to be somewhat less important than smut, yet it is quite destructive to certain selfed lines of corn. While it would be desirable to ascertain the reaction of selfed lines to the different physiologic forms of *P. sorghi* present in the locality, it seems probable that, by careful selection for resistance to rust during the several years of selection in self-fertilized lines, and by close observation of the lines selected for use in the production of crosses or synthesized varieties, those biotypes which are highly susceptible to the rust forms present can be eliminated with a fair degree of success. The facts presented indicate the necessity of careful selection for rust resistance either under artificially induced or natural epidemic conditions so that any new varieties produced will be at least as resistant as was the original variety from which the inbred lines were developed.

SUMMARY

1. *Puccinia sorghi* Schw. may become a destructive pathogene on newly developed lines of corn. It caused considerable damage to certain inbred lines of corn grown at University Farm, St. Paul, in 1923 and in 1925.

2. Notes were taken on the rust and smut reaction of 171 selfed lines of corn. Some lines were extremely susceptible, others highly resistant, while still others were intermediate in their reaction to rust.

3. All combinations of resistance and susceptibility to corn rust and to corn smut appeared in the field.

4. Collections of *P. sorghi* were obtained from many different places in the United States and one place in Canada. Forty-five selfed lines of corn were inoculated with most of these collections.

5. Seven physiologic forms of *P. sorghi* can be recognized by their parasitic behavior on eight selfed lines of corn. Two forms were isolated from Minnesota, and one each from the following states: Texas, Oklahoma, Kansas, Nebraska, and Iowa.

6. Form 1, besides being relatively weak in pathogenicity, also produced uredinia decidedly lighter in color than those of the other six physiologic forms. It never produced telia, while other forms produced them readily.

7. The fact that there are numerous physiologic forms of *P. sorghi*, some of which are quite injurious to certain selfed lines of corn, should be taken into consideration in breeding work.

8. It is important in producing new varieties of crop plants to determine their reaction to physiologic forms of the most important pathogenes

and to take cognizance of the fact that new varieties or lines may be far more susceptible to hitherto unimportant pathogenes than varieties now commonly grown.

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THE FUNGICIDAL ACTION OF LIQUID LIME SULFUR

M. C. GOLDSWORTHY[1]

Lime-sulfur solutions have been known and used as fungicides for a long time. Lodeman (5, p. 16) reports their use as early as 1852. It was not until 1906, however, that this fungicide and insecticide came into extensive use in the United States. At that time Cordley (1), in Oregon, introduced the material for fungicidal and insecticidal purposes.

The fungicidal properties of lime-sulfur solutions have been a topic of wide interest. It has been the opinion of most investigators that the fungicidal value of liquid lime-sulfur is indirect, and that finely divided sulfur or some oxidation product of the same, which is liberated on exposure to the oxygen of the air, is the toxic substance.

Foreman (4), in 1910, ascribed the fungicidal properties of liquid lime-sulfur to the alkalinity expressed by such solutions. Eyre and Salmon (2), in 1916, reported the results of investigations on the fungicidal properties of spray liquids. During this work they found that the fungicidal properties of lime-sulfur solutions were entirely a function of the soluble polysulfide content. They proved to their satisfaction that the alkalinity of the solutions was not responsible for their fungicidal properties. They compared, quite exhaustively, the effects of various sulfide mixtures. Their criterion of toxicity was based upon the effect of the solutions upon the growth of superficial patches of the mycelium, conidiophores, and conidia of the powdery mildew fungus, *Sphaerotheca humuli* (D. C.) Burr. They made a point of the fact that only patches of the same age could be used. Eyre, Salmon, and Wormald (3), in 1919, continuing the experiments on lime-sulfur, again came to the conclusion that the fungicidal properties of this material were due to the amounts of sulfides in solution. They found that sodium thiosulphate was ineffective in controlling the fungus. During this work, they found that one of their sulfide solutions which contained a great deal of finely divided sulfur, indicating the breaking down of the sulfide, was just as effective as one in which the sulfide had not been thus affected. As the content of soluble sulfide had been reduced beyond that which was found to be effective, they attributed the toxicity of the solution containing sulfur to both constituents. They left the subject of the toxic properties of lime-sulfur solutions still in doubt.

In 1922, Young (7) published on the toxic properties of sulfur. Quoting many researches and advancing considerable data himself, he was of the

[1] Contribution from the Division of Plant Pathology, University of California.

opinion that a relation existed between toxicity and an oxidation product of sulfur, namely, pentathionic acid. He regarded the effect of liquid lime-sulfur as being due to the oxidation of the sulfides to finely divided sulfur, which is, in turn, oxidized to some higher form which acts as the toxic substance. He found that calcium thiosulphate had no value as a fungicide, thereby supporting Eyre in this respect. He did not regard the sulfide ion as the toxic substance, but based his whole theory upon the oxidation products of elemental sulfur.

In England, during the meeting of the Economic Botanists in 1926, the matter of the toxic effect of sulfur was thoroughly discussed at a symposium on the subject (6). The fungicidal properties of the polysulfide solution were attributed to the direct action of sulfur which is rapidly formed by the decomposition of the sulfide in the presence of oxygen. This toxicity, it was claimed, could be shown by bringing plants infected with mildew into an atmosphere which contained heated sulfur. If the finely divided sulfur had been screened from the chamber toxicity was not demonstrated. The hypothesis of Young was held untenable on account of the fact that toxicity is demonstrated at hydrogen ion concentrations which are inhibitive to the formation of pentathionic acid.

EXPERIMENTAL DATA

In connection with laboratory studies on the peach rust fungus, *Tranzschelia punctata* (Pers.) Arth., a variety of fungicides was tested. Liquid lime-sulfur solutions, diluted as high as one to a hundred, gave excellent results.

During observation with the microscope of germinating urediniospores which had been sprayed with liquid lime-sulfur solutions, it was noticed that the lumina of the germ tubes became more or less filled instantaneously with what appeared to be sulfur globules, which resembled those appearing upon the slides from the oxidation of the sulfide solutions. Many observations were made, always with the same results. Whenever the germ tubes came in contact with the solution, the globules were formed. This is shown quite well in Plate IV, A. The germ tube can be seen in an area which formerly was a drop of lime-sulfur solution, where sulfide has changed to sulfur globules. Within the germ tube similar globules are seen, especially near the lime-sulfur drop. At this stage it is difficult to differentiate between the globules within the germ tubes and those on the slide. The question which naturally arose was, were these globules within the tubes or were they on the surface of the spores and tubes. It became necessary to demonstrate in some manner the actual presence of the globules within the tubes and to ascertain if they were, as the writer suspected, sulfur globules. That this is the case was proved by the following method.

In some preliminary studies, it was found that the urediniospores of this fungus would not germinate in drops of tap or distilled water. It was noted also that spores which were scraped off of the sori would not germinate. Only those which were shaken off germinated readily. From all indications it was purely a matter of maturity, those spores which were easily shaken from the sori being the mature ones. The spores germinated only in an atmosphere which was saturated. Accordingly, the method used for the germination of the spores was as follows: the spores were dusted or shaken on to slides; the slides containing the spores were then placed in a saturated atmosphere at room temperature. In 12 hours the germ tubes reached their maximum growth, at which time the germinated and ungerminated spores were sprayed with the desired strength of lime-sulfur solution. This was accomplished by spraying with an atomizer.

After considerable effort, the problem of dissolving away the sulfur on the slides without interfering with that within the germ tubes was solved. The method is quite simple, once worked out. The steps in the method are as follows. The slide is washed with dilute hydrochloric acid to remove the carbonates and thiosulphates which, as well as sulfur, remain upon the slide. After the treated slide has been washed with the acid, it is cleaned off with distilled water. The slide is dried by slightly warming in a flame, and, while warm, flooded with carbon tetrachloride; this dissolves the sulfur which remains on the slide. For some reason, possibly because the sulfur inside the tubes and spores is fixed within a protoplasmic mass or is surrounded by water, the tetrachloride does not penetrate into these parts. The sulfur of the outside system is removed by this method. Flooding a few times removes all of the sulfur from the slide and surfaces of the spores and tubes. After the tetrachloride has evaporated, a few drops of sodium hydroxide, N/5 solution, are placed on the slide. The hydroxide solution appears to penetrate into the cells and slowly reduces the elemental sulfur to sulfide ion. The sulfide ion can then be demonstrated by placing a few drops of sodium nitroprusside along with the hydroxide. The globules within the tubes and spores are now a deep violet color, which is indicative of the exchange of the NO part of the nitroprusside ion with the sulfide ion from the globules. The plastic globules are in this manner shown to be elemental sulfur. Plate IV, B shows the globules after having been treated with the hydroxide and nitroprusside ion. The sulfur within the spores does not show very well. Under the microscope, however, these are deep violet in color.

The same technique is as satisfactory for sprayed spores. Spores and germinating spores which have not been sprayed and which do not contain the globules do not give the nitroprusside reaction when hydroxide is allowed to diffuse into the cells. Liquid lime-sulfur, when allowed to dry

with the resultant liberation of plastic sulfur, reacts, giving the same violet color as obtained within the tubes. Finely divided elemental sulfur, when acted upon by strong sodium hydroxide solutions, gives the same reaction with nitroprusside.

It is evident from this demonstration that the globules within the germ tubes and spores are plastic sulfur. By the presence of sulfur within the spores and tubes one comes to the conclusion that the sulfide which had been sprayed on them had entered the cell and had been oxidized to some form of elemental sulfur. The oxidation of sulfide to sulfur, probably by direct reduction of the protoplasm, apparently interferes with the growth of the germ tubes, and growth stops. Many observations with spores and germinated spores were made relative to this point. Table 1 shows the effect of liquid

TABLE 1.—*Effect of liquid lime-sulfur, 1–100, on the length of germ tubes of urediniospores of Tranzschelia punctata.*

Germ tube	Time sprayed	Length in microns after			
		3 hours	6 hours	9 hours	12 hours
1	Third hour	6	6	6	6
2	do	8	8	8	8
3	do	6	6	6	6
4	Sixth hour		17.5	17.5	17.5
5	do		23.0	23.0	23.0
6	do		15.0	15.0	15.0
7	Ninth hour			38.0	38.0
8	do			46.5	46.5
9	do			35.0	35.0
10	Not sprayed				61.5
11	do				82.0
12	do				77.0
13	do[a]				71.5

[a] Average of 12 germ tubes.

lime-sulfur solution (diluted 1 part to 100 parts of water) on the length of the germ tube. Table 2 shows the effect of various sulfur compounds upon the germinability of urediniospores. Apparently the limit of laboratory control with liquid lime solutions lies near the dilution of 1–100. A few of the spores germinate at this dilution, while dilutions less than this give perfect results.

It is evident, from the data presented, that an injurious effect on the metabolism of the host results from the application of various solutions of liquid lime-sulfur. The formation of sulfur from sulfide brings about an irreversible change in the oxidation-reduction system of the cell. The in-

TABLE 2.—*Effect of sulfur compounds upon the germinability of urediniospores of Tranzschelia punctata*

Compound and dilution	Temperature	No. spores counted	No. spores germinating	Percentage of germination
Commercial dry lime sulfur 14 lbs.–100 gals. H_2O	22° C.	4036	242	6.0
Liquid lime-sulfur, 1–100	do	1376	12	0.9
Liquid lime-sulfur, 1–50	do	3001	0	0.0
Liquid lime-sulfur, 1–10	do	3378	0	0.0
Powdered sulfur (old lot)	do	2060	198	9.1
Powdered sulfur (new lot)	do	8336	118	1.4
Sulfur paste, gas house residue	do	1723	49	2.8
No treatment	do	12,425	3516	28.3

jury to the system can be interpreted in the light of the oxidation of the sulfide to sulfur through the agencies of the cell.

DISCUSSION

The fungicidal properties of polysulfide solutions are dependent, at least in part, upon its soluble sulfide content. The theory of Young and others—that polysulfide solutions act only when the oxidation product of the resultant liberated sulfur is formed—seems unnecessary in view of the observations and data of the writer.

The fact that the formation of sulfur within the germ tubes represents an instantaneous reaction, and that the germ tubes and spores are arrested in their development, forces one to believe that the oxidation of the sulfide to sulfur is the lethal factor. In the light of the development of knowledge regarding the oxidation-reduction systems and of the hydrogen-ion relations of cells, one can better understand the effect of sulfide solutions upon the protoplasm of the germ tubes and spores. The absorption of sulfide solutions brings about at least two changes: (1) a reduction of the oxidation-reduction potential, and (2) of the hydrogen ion concentrations. Either one in itself, if carried far enough, would be responsible for an irreversible change in the poise of the protoplasmic system. It is hardly possible that the small amounts of sulfide solutions which were used could bring about such an irreversible change principally on account of hydrogen ion relations. It seems most probable that the poising material of the oxidation-reduction system has been used up to a point beyond which recovery is impossible. Since the formation of elemental sulfur is coincident with, or followed by, death of the organism, the logical conclusion is that this sub-

stance represents the end product of a reaction concerned with the oxidative properties of the protoplasm of the cell, probably influenced, in part but not entirely, by the alkalinity of the solution.

These results confirm those of Eyre and Salmon upon the effect of liquid lime-sulfur solutions—that the toxic properties of sulfide solutions are functions of their respective soluble sulfide content.

SUMMARY

1. Liquid lime-sulfur solutions are instantaneously reacted upon by the protoplasm of the urediniospores and germ tubes of the peach rust fungus, *Tranzschelia punctata* (Pers.) Arth.

2. Globules of plastic sulfur are formed within the lumina of the germ tubes and within the spore cover.

3. A method for the determination of plastic sulfur within the germ tubes and spores is presented.

4. The reduction of the protoplasm of the host, through the oxidation of polysulfide to elemental sulfur, appears to be an important factor in the fungicidal action of liquid lime-sulfur, as determined by its effect upon the growth of germ tubes and upon the germinability of spores.

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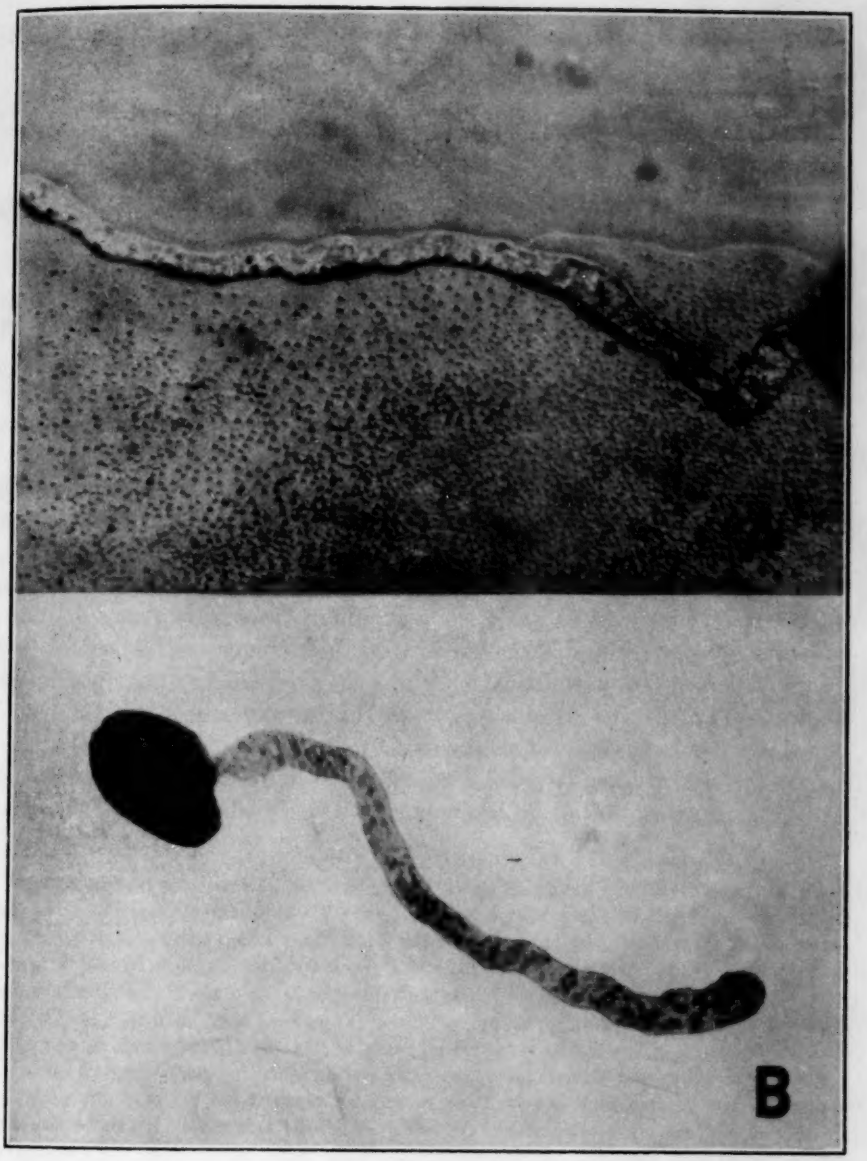
EXPLANATION OF PLATE IV

A. Germ tube of urediniospore. Sprayed with lime-sulfur 1–100 dilution and showing the sulfur globules on the slide and within the germ tube.

B. Germ tube of urediniospore. Sprayed with lime-sulfur 1–100 dilution and showing the sulfur globules within the germ tube after treatment with sodium hydroxide and nitroprusside ion. ×940.



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COMPARATIVE VIRULENCE OF CERTAIN STRAINS OF PYTHIUM IN DIRECT INOCULATION OF CONIFERS[1]

E. J. ELIASON

INTRODUCTION

Many of the reports of the casual relationships between the different damping-off fungi and their hosts have been based upon the presence of fungi in diseased seedlings rather than upon inoculations with them. Hartley (4) demonstrated by inoculation that the various strains of the same species differed in their ability to cause damping-off of coniferous seedlings. Edson (1) demonstrated also that only part of the *Corticium vagum* strains isolated from sugar beets were able to attack that host vigorously. Gilbert (2) found by repeated inoculations that some strains of *Cladosporium cucumerinum* were uniformaly virulent to cucumbers while others failed to produce infection. Because of this strain variability it is entirely possible that a saprophytic strain of a species which is usually parasitic may be associated with a damped-off seedling or that a parasitic strain of some damping-off fungus may be living as a saprophyte in a seedling damped-off by some other fungus. Therefore it is necessary to test the pathogenicity of damping-off fungi by inoculation experiments.

The object of the present investigation was to test the parasitism of certain Pythium strains and other fungi which had never before been tested on coniferous seedlings, and to determine their virulence in relation to one another and to other fungi used as standards by Rathbun-Gravatt (7). A list of the fungi tested is given in table 1.

[1] This article is a part of a thesis presented to the faculty of the New York State College of Forestry, Syracuse, N. Y., for the partial fulfillment of the requirements for the degree of Master of Science. The work herein reported was done in partial cooperation with the Office of Forest Pathology, United States Department of Agriculture, and is a continuation of some of the inoculation experiments which were started by that office. The *Pythium debaryanum* (?) cultures isolated from coniferous hosts were secured from that office. They are of the type formerly considered as *Pythium debaryanum* but in the present uncertain state of knowledge concerning this group it is possible that some of them belong to other species. The Pythium strains from angiospermous hosts were sent by Dr. Charles Drechsler, Office of Vegetable and Forage Diseases, U. S. Department of Agriculture. The culture of *Aphanomyces euteiches* was furnished by Dr. F. R. Jones, of Wisconsin. The writer is indebted to Mrs. A. R. Gravatt, Dr. Carl Hartley, and Dr. L. H. Pennington for their kindly criticisms and suggestions.

TABLE 1.—*Identity and source of the fungi tested*

Name	Strain no.	Source		
		Host	Locality	Isolator
Alternaria sp.		*Pinus resinosa*	New York	E. J. Eliason
Aphanomyces euteiches		*Pisum sativum* (root)	Wisconsin	F. R. Jones
Corticium vagum	147[a]	*Picea engelmanni*	Washington, D. C.	C. Hartley
Fusarium moniliforme	249[a]	*Pinus ponderosa*	Kansas	T. C. Merrill
Fusarium sp.		*Pseudotsuga taxifolia*	New York	E. J. Eliason
Pythium debaryanum	296[b]	*Beta vulgaris*	Wisconsin	H. A. Edson
P. debaryanum (?)	1036	*Pinus banksiana*	Nebraska	G. G. Hahn
do	1040	*Pinus sylvestris*	Virginia	do
do	1047	do	do	do
do	1071	do	do	A. R. Gravatt
do	1072	do	do	do
do	1074	do	do	do
do	1080	*Pinus taeda*	do	do
do	1084	*Pinus sylvestris*	do	do
do	1086	do	do	do
do	1091	*Pseudotsuga taxifolia*	Colorado	G. G. Hahn
do	1092	do	do	do
do	1093	do	do	do
do	1094	do	do	do
do	1095	do	do	do
P. debaryanum	1E (Jones no. 662) 1922	*Pisum sativum* (root)	Wisconsin	F. R. Jones
Pythium sp.	2E	do	New York	C. Drechsler
do	3E (spiny)	do	Georgia ?	do
P. aphanidermatum	4E	*Nicotiana tabacum*	Africa	Dade-Buller
Pythium sp.	5E	*Cucumis sativa*	Illinois	.....
do	8E (Jones no. 830–1)	*Avena sativa* (root)	Wisconsin	F. R. Jones
P. debaryanum	9E	*Citrullus vulgaris*	Virginia	C. Drechsler

[a] Used in inoculation experiments by Hartley et al (3, 4) and by Rathbun-Gravatt (7).

[b] Used by Edson (1).

METHODS OF INOCULATION

In the writer's experiments three methods of direct inoculation were used, all of which have been described by Rathbun (6). Throughout this paper these methods will be designated as (a) "platform method," (b) "petri dish method 1," and (c) "petri dish method 2." The "platform method," in which the seedlings were inoculated about 2 cm. above the soil surface, was slightly modified: the seedlings were not grown in glass cupboards, and bell jars instead of individual celluloid cones were used as damp chambers. In the "petri dish method 1," sterile seedlings were placed in petri dishes, the bottoms of which had been covered with moist filter paper, and inoculum placed on any selected portion. In "petri dish method 2,"

TABLE 2.—*Results of inoculating the stems of Pinus resinosa with four strains of Pythium debaryanum (?) by the platform method*

Strain no.	No. seedlings inoculated	No. seedlings in contact with inoculum at end of experiment[a]	Seedlings damped-off		
			No.	Percentage of total	Percentage of those in contact
1091	40	20	7	18	35
1092	40	21	14	35	67
1093	40	27	4	10	15
1094	40	27	14	35	52
Control	100	56	0	0	0

[a] Because of excessive drying of the sand the inoculum was not in contact with part of the seedlings at the end of the experiment.

TABLE 3.—*Results of inoculating, by the platform method, stems of Pseudotsuga taxifolia with strains of Pythium debaryanum (?) reisolated from the Pinus ponderosa stems used in the previous experiment (table 2)*

Strain no.	No. seedlings inoculated in each series	Seedlings damped-off			
		Series I		Series II	
		No.	Percentage	No.	Percentage
1091	20	0	0	0	0
1092	20	2	10	6	30
1093	20	1	5	1	5
1094	20	3	15	2	10
1095[a]	20	3	15	1	5
Control	20	0	0	0	0

[a] Original strain not used in previous experiments.

TABLE 4.—*Results of inoculating Pinus strobus stems with the five original strains of Pythium debaryanum (?) and with other fungi by the platform method*

Fungus	Strain no.	No. seedlings inoculated	Seedlings damped-off	
			No.	Percentage
Aphanomyces euteiches		8	0	0
Corticium vagum	147	7	5	71
Fusarium moniliforme	249	7	1	14
Fusarium sp.		8	0	0
Pythium debaryanum (?)	1091	8	1	12
do	1092	11	1	9
do	1093	8	4	50
do	1094	7	7	100
do	1095	12	0	0
Control		20	0	0

TABLE 5.—*Results of inoculating Pinus banksiana stems with three reisolated[a] strains of Pythium debaryanum (?) by petri dish method 1*

Strain no.	No. seedlings inoculated	Seedlings damped-off	
		No.	Percentage
1091	10	2	20
1092	10	6	60
1093	10	4	40
Control	10	0	0

[a] Same strains as referred to in table 3. Originally from *Pseudotsuga taxifolia* and reisolated from *Pinus resinosa*.

sterile medium was inoculated with the desired fungus, and after the mycelium had completely covered the surface, a cut was made across the center of the dish and all the material on one side of the cut removed. The root or stem of the seedling was then placed upon the medium remaining in the petri dish.

In each experiment, control plants were treated in exactly the same manner as the others except that sterile agar was used instead of agar inoculated with fungus. All the fungi were grown on cornmeal agar made according to the formula used by the Shear and Stevens (8) for Endothia cultures.

All the inoculations referred to in any one table were made at the same time. In the first experiment, of which the results are given in table 2, the temperature varied from 15° to 25° C. For all the others the temperature was kept fairly constant at about 21° C.

TABLE 6.—*The rank in virulence of five different strains of Pythium debaryanum (?) on four host plants. Summary of results of tables 2–5*

Strain no.	Rank in virulence on				
	Pinus resinosa	*Pseudotsuga taxifolia*		*Pinus strobus*	*Pinus banksiana*
		Series I	Series II		
1091	3	5.0	5.0	3	3
1092	1	3.0	1.0	4	1
1093	4	4.0	3.5	2	2
1094	2	1.5	2.0	1	....
1095	—	1.5	3.5	5	....

TABLE 7.—*Results of inoculating Pseudotsuga taxifolia stems with strains of Pythium debaryanum (?) and with other organisms by the platform method*

Fungus	Strain no.	No. seedlings inoculated	Seedlings damped-off	
			No.	Percentage
Alternaria sp.		16	0	0
Aphanomyces euteiches		14	2	14
Corticum vagum	147	19	5	26
Fusarium sp.		15	0	0
Fusarium moniliforme	249	14	4	29
Pythium debaryanum (?)	296	16	5	31
do	1036	16	4	25
do	1040	17	6	35
do	1047	16	1	6
do	1071	15	5	33
do	1072	17	0	0
do	1074	15	3	20
do	1080	16	7	44
do	1084	17	3	18
do	1086	14	0	0
do	1094	16	3	19
Control		16	0	0

EXPERIMENTAL RESULTS

The results of all of the experiments are given in tabular form (Tables 2 to 10). The data given in tables 2 to 5 inclusive are summarized in table 6.

TABLE 8.—*Results of inoculating the stems of various coniferous hosts with various damping-off fungi by the platform method*

Fungus	Host	No. seedlings inoculated	Seedlings damped-off	
			No.	Percentage
Alternaria sp.	*Pseudotsuga taxifolia*	17	0	0
Aphanomyces euteiches	do	17	2	12
Corticium vagum	*Pinus banksiana*	16	11	69
do	*Pseudotsuga taxifolia*	16	9	56
Fusarium moniliforme (249)	do	16	0	0
do	*Pinus ponderosa*	6	0	0
Pythium debaryanum, No. 1094 (Original)	*Pseudotsuga taxifolia*	17	0	0
Control		30	0	0

TABLE 9.—*Results of inoculating roots of coniferous seedlings with Aphanomyces euteiches by the petri dish methods*

Host	No. seedlings inoculated by each method	Seedlings with rotted roots			
		Petri dish method 1		Petri dish method 2	
		No.	Percentage	No.	Percentage
Picea engelmanni	5	3	60	2	40
Pinus banksiana	5	1	20	0	0
P. ponderosa	5	0	0	0	0
P. strobus	5	0	0	0	0
Pseudotsuga taxifolia	5	0	0	0	0
Control	5	0	0	0	0

TABLE 10.—*Results of inoculating, by the platform method, stems of Pseudotsuga taxifolia with strains and species of Pythium isolated from angiospermous hosts*

Fungus	Strain no.	No. seedlings inoculated	Seedlings damped-off	
			No.	Percentage
Pythium debaryanum (?)	1E	11	2	18
Pythium sp.	2E	9	4	44
do	3E	10	2	20
P. aphanidermatum	4E	11	3	27
Pythium sp.	5E	9	1	11
do	8E	12	3	25
P. debaryanum	9E	12	5	42
Control		25	1	4

SUMMARY

1. The experimental data show that, of the 22 species and strains of *Pythium* tested, all but three caused damping-off in some coniferous seedlings.

2. *Aphanomyces euteiches*, a parasite upon roots of *Pisum sativum*, caused damping-off and root rot in a few coniferous seedlings.

3. An *Alternaria* and a *Fusarium* isolated from coniferous material gave no evidence of parasitism in these experiments.

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THE RELATION OF TEMPERATURE DURING THE GROWING SEASON IN THE SPRING WHEAT AREA OF THE UNITED STATES TO THE OCCURRENCE OF STEM RUST EPIDEMICS

E. C. Stakman and E. B. Lambert[1]

Black stem rust is one of the typically epidemic diseases of plants. In the United States, the greatest variation in the severity of attack is in the upper Mississippi Valley, particularly in the area growing hard red spring wheat. In some years the rust causes relatively little damage; in others only a moderate amount; whereas in still others it develops devastating epidemics which must be experienced to be appreciated. The apparent suddenness of onset and the amazing rapidity of spread have driven many farmers into bankruptcy and many pathologists well nigh to distraction.

Obviously, several factors must operate in proper sequence and conjunction in order that an epidemic may develop. First of all, there must be abundant inoculum, favorable conditions for its dissemination and the germination of the spores, the entrance of the germ tubes into the plants, the subsequent rapid development within the plants, and the production of still more inoculum. There also must be a fairly dense and widespread population of susceptible hosts.

The first of the requirements usually is satisfied because the number of spores can multiply so rapidly. There are two sources of initial inoculum in the hard red spring wheat area: (1) aeciospores from the common barberry, and (2) urediniospores which may be blown in from the South. There usually is plenty of wind to disseminate the spores. Except in unusually dry years, dews are likely to be sufficiently heavy and frequent, even in the absence of frequent rains, to promote germination of the spores and entrance of the germ tubes; there are many susceptible varieties of small grains and wild grasses; there is practically always sufficient light for the development of the rust; but the temperature varies considerably in different seasons.

It has been shown by many observations and controlled experiments that light and temperature affect the development of the rust on inoculated

[1] Cooperative investigations between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Minnesota Agricultural Experiment Station.

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plants more than any other environmental factors. Predisposition of the host does not seem to be very important, as plants growing under fairly normal conditions are sufficiently susceptible to enable the rust to develop well.

The amount of sunlight, of course, varies in different years, but usually not enough to affect very profoundly the development of rust. This leaves temperature as one of the important variables in the environment which determine whether or not epidemics will develop.

Opinions regarding the effect of temperature on the development of rust have differed considerably. The controlled experiments of Melhus, Durrell, and Kirby (4), Stakman and Levine (7), Peltier (6), and Johnson (3) have given us pertinent facts. They indicate a profound influence of temperature on the germination of teliospores, the germination and longevity of urediniospores, and the rate of development of the uredinial stage. But the results of these studies on stem rust can not directly explain the development of epidemics. The rate of development of the host and the factors affecting it must be considered also, because in epidemic years there is a race between the pathogene and the host plant. One would expect the development of epidemics to be favored by cool weather because of the delayed maturity of the host. Freeman and Johnson (2) pointed out that such conditions favored the development of the epidemic in 1904. However, Blair (1) has shown that yields usually are higher in cool seasons than in hot ones. Therefore, it seemed desirable to determine whether there is a correlation between temperature during the growing season and the development of rust epidemics.

The historical method, so successfully used by Martin (5) in studying the relation of weather to epidemics of late blight of potato in New Jersey, also was used in the present study of the effect of temperature on the development of stem rust.

The average temperature prevalent during the growing season in epidemic years is compared with that for non-epidemic years from 1904 to 1925, inclusive. Barberries usually become rusted in May. The late spring and early summer months seem to be the critical time for the development of rust in the hard red spring wheat area. This period involves the development of the barberry leaves, the germination of teliospores, the dissemination of sporidia, the infection of the barberry, subsequent discharge of the aeciospores, and the beginning of local epidemics near barberry bushes. Spring wheat usually is sown in April, and its development is considerably affected by temperatures during May. During May, June, and July, the rust usually spreads from the barberries and multiplies in the uredinial stage. Wheat also is making its principal growth during June and July. After the first week in August, wheat and other small grains

usually are mature or so nearly so that rust does not develop on them. It is possible also that the temperature during the fall and winter may affect the development of rust by influencing overwintering of the uredinial stage and the production and maturity of teliospores. However, the factors affecting the production and maturation of teliospores are obscure, and the uredinial stage of the rust seldom, if ever, overwinters to any considerable extent in the spring wheat area. May, June, and July therefore were selected for study.

EPIDEMIC, INTERMEDIATE, AND NON-EPIDEMIC YEARS

There have been at least six destructive epidemics in the hard red spring wheat area during the past quarter of a century. Personal observations and the records of the U. S. Plant Disease Survey from 1904 to 1925 indicate that the worst epidemics occurred in 1904, 1911, 1916, 1919, 1920, and 1923. The following could be classed as intermediate years: 1905, 1906, 1908, 1914, 1917, 1921, 1922, and 1925. There was comparatively little rust in 1907, 1909, 1910, 1912, 1913, 1915, 1918, and 1924. The first series will be designated epidemic years, the second intermediate, and the third non-epidemic.

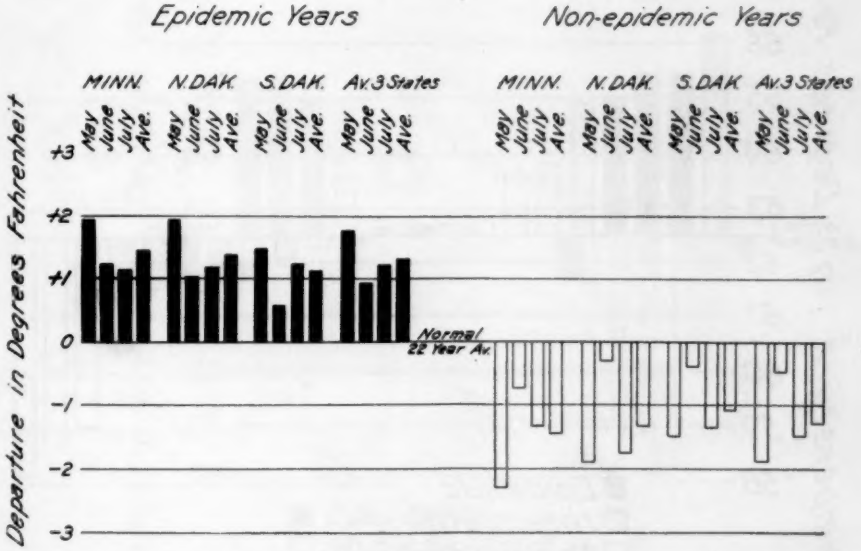

FIG. 1. A comparison of the departure from the normal of the average of the mean monthly temperature of all the epidemic years, with the average of all the non-epidemic years, for Minnesota, North Dakota, and South Dakota, in May, June, and July, from 1904 to 1925, inclusive.

TEMPERATURE AND EPIDEMICS

The average mean monthly temperatures during May, June, and July in Minnesota, North Dakota, and South Dakota for the epidemic years was compared with that for the non-epidemic years. As shown in figure 1, the average temperature throughout the growing season in all three states was consistently above normal in the epidemic years and below normal in the non-epidemic years.

The amount of departure from the normal for the 22-year period also was studied. As shown in figure 2, the three coolest years were non-epidemic years and the five warmest years were either epidemic or intermediate years. When the epidemic and non-epidemic years are considered separately, as in figure 3, it becomes apparent that destructive epidemics did not develop in seasons during which the average temperature was below 61° F. Nor were there any non-epidemic seasons when the average tem-

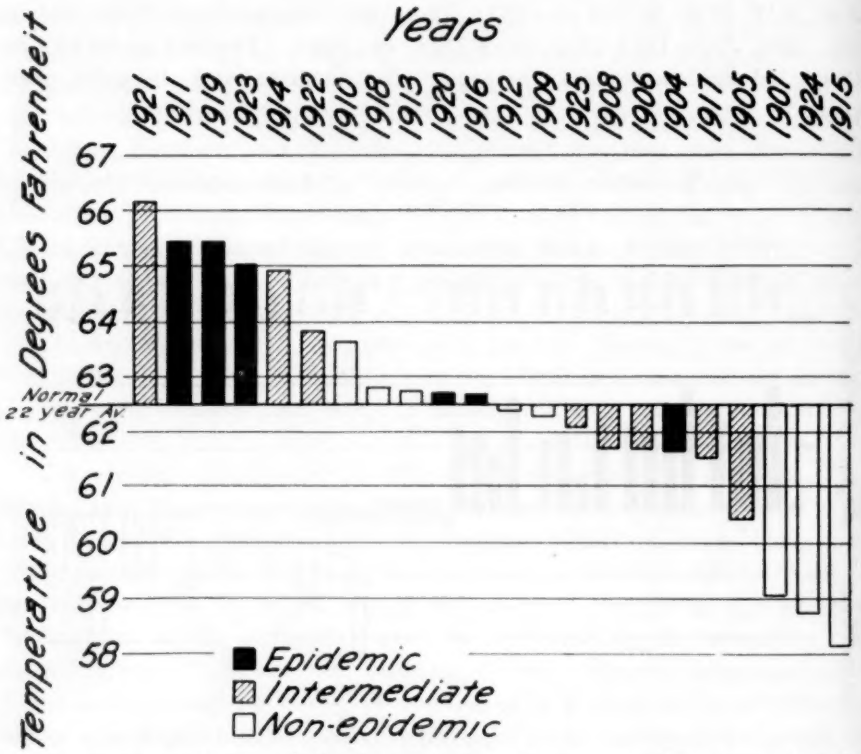

FIG. 2. A comparison of the departure from the normal of the average of the mean monthly temperatures for the growing season (May, June, and July) in the spring wheat area (Minnesota, North Dakota, and South Dakota) from 1904 to 1925, inclusive.

perature was above 64° F. There were both epidemic and non-epidemic years when the average temperatures were intermediate (61–64° F.). This is exactly what one would expect on the assumption that temperature is only one of many interdependent factors influencing the development of epidemics.

The weather conditions in three of the years with moderate temperatures are especially interesting: in 1910, because so little rust developed in spite of a comparatively high average temperature; and in 1916 and 1904, because very destructive epidemics developed when the temperature was moderate. In 1910, precipitation clearly was the limiting factor. The average precipitation was less than two inches for each of the three months under consideration in each of the three states, with the exception of June in South Dakota, when there was an average precipitation of 2.27 inches. There was such a decided and protracted drought that infection was practically impossible. It was the only year out of 19 when at least a moder-

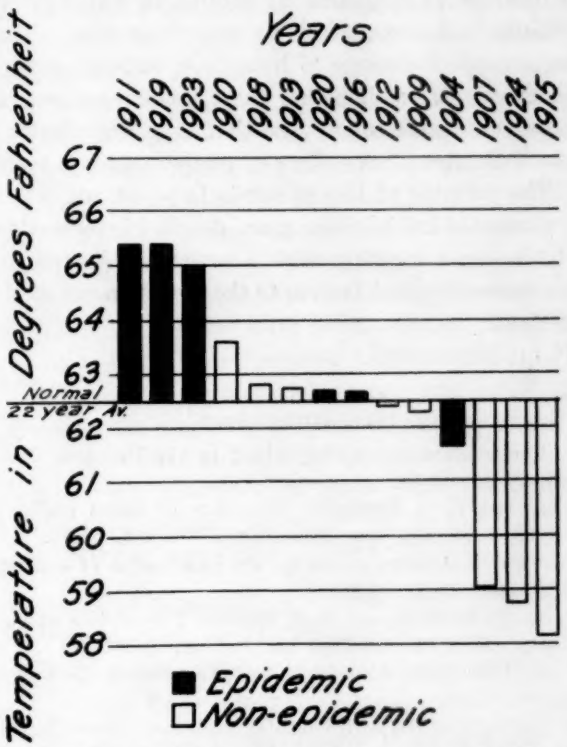

FIG. 3. A comparison of the departure from the normal of the averages of the mean monthly temperatures for the growing season (May, June, and July) in the spring wheat area (Minnesota, North Dakota, and South Dakota) which occurred during the stem rust epidemic years and non-epidemic years from 1904 to 1925, inclusive.

ately severe epidemic did not develop in the artificially inoculated rust nursery at University Farm, St. Paul, Minnesota. The epidemic of 1916 was the most destructive on record. This may seem surprising when one notes that the average temperature was but little above normal. But the average temperature during July was higher in all three states than that of any other year under consideration. Not only did the intense heat aggravate the damage from rust, but it undoubtedly was directly responsible for much of the injury popularly attributed to rust.

It is difficult to account for the epidemic of 1904. The temperature was lower than normal and the rainfall was not excessive. It is evident, therefore, that epidemics may develop even in moderately cool seasons, although this seems to be the exception rather than the rule.

CONCLUSIONS

From the data presented, it is evident that, in the past, there has been a tendency for destructive epidemics to develop in warm growing seasons, and for cool seasons to be comparatively free from rust. The amount of departure from normal also seems to have been correlated with the occurrence of epidemics. It is possible that these tendencies were coincidences, but it seems more reasonable to suppose that they represent a causal relationship and are indicative of the effect of temperature on the development of epidemics. The purpose of this paper is to point out this relationship.

The graphs presented are based on more detailed information, which will be given in a later paper together with a report of similar studies on the relation of other meteorological factors to the development of stem rust.

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